EFFICACY OF TETRAZOLIUM TEST FOR EVALUATING SEED VIABILITY OF MAJOR PULSE CROPS

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Affectionately Dedicated to

My Beloved Parents

DEPARTMENT OF SEED TECHNOLOGY
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CERTIFICATE

This is to certify that the thesis entitled "EFFICACY OF TETRAZOLIUM TEST FOR EVALUATING SEED VIABILITY OF MAJOR PULSE CROPS" submitted by Mr. UMESHA, K., for the degree of MASTER OF SCIENCE (AGRICULTURE) in SEED TECHNOLOGY of the University of Agricultural Sciences, Bangalore is a record of bona fide research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
March 15 ,1995

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[UMESHA. K]

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LIST OF ABBREVIATIONS

BP	Between paper
h	Hour
g	Grams
ml	Milli litre
mm	Milli metre
min.	Minutes
TZ	Tetrazolium
TTC	Triphenyl Tetrazolium chloride
TP	Top of paper
V/V	volume/volume

INTRODUCTION

I. INTRODUCTION

Pulses form an important part of Indian dietary. They are an important source of protein to those who are predominantly the vegetarians. The total annual area under pulse crops in India is about 22 million hectares and the production is 12 million tonnes of grain (Anon., 1987). In Karnataka pulses are grown over an area of 17.18 lakh hectares with 8.30 lakh tonnes of production and the productivity is around 450 to 460 kg per hectare (Anon., 1994).

Seed testing in pulses is one of the important steps in regulating the quality of seeds being produced and marketed. Use of poor quality seed is one of the reason for low seed yield and lower productivity of pulses in India. Hence, it is essential to improve the quality of seeds by routine tests like germination tests before they are used for sowing or marketing. The total seed requirement of pulses in Karnataka is 4.88 lakh quintals including 0.54 lakh quintals of certified seeds (Anon., 1994).

Germination test is the best indicator of the planting value of a seed lot to emerge under field conditions. However, the germination test takes more time in some of the legume crops due to embryo or seed coat dormancies. Further, whenever seed lots are to be despatched to long distance within a short period of time, there is a need to apply quick viability tests, which are usually the biochemical tests used for both dormant and non-dormant seeds. One of the most reliable technique is the use of 2, 3, 5-Triphenyl tetrazolium chloride for the assessment of seed viability.

The practical utility of tetrazolium test for seed viability has been realized by the seed scientists and technologists all over the world. The development of tetrazolium test is the most significant advance in seed testing technology of this century (Anon., 1970). The method was pioneered by the German Scientist Lakon during the second war era (1939-1958), who recoginzed that all living tissues, which respire, are capable of reducing the colourless chemical. 2,3,5,-triphenyl tetrazolium chloride or bromide into a red coloured, stable and non-diffusable compound called Formazan.

The technique involves imbibition of a seed in a solution of colourless TZ solution having the pH of 6.0 to 7.0. In the viable tissues, the solution is reduced by dehydrogenase enzymes to Formazan. The judgement on the ability of a seed to germinate is based on the pattern and degree to which the embryo is stained (Mackay, 1972). Since the tissues within a seed could be at different states of viability, they would be stained differently. Moore (1973) described the use of TZ staining more efficiently on the basis of topographical staining patterns of seeds. Further, the tetrazolium test has been developed to furnish quick estimates of seed germinability. This test is very useful for those who require results in a short time for taking timely decisions in seed processing, storing and marketing situations. TZ test has the advantage of testing dormant seed lots, vigour rating of seed lots and to detect the cause for seed deterioration.

Tetrazolium test is not easy to perform unless the test methodologies have been standardized and the analyst should have sufficient experience in evaluation work. During the present investigation, the main emphasis was focussed on the use of low dilutions of TZ solutions to reduce the cost factor and also to reduce the time consumed for steps like preparation time, reaction or incubation time etc., in six major pulse crops. The results generated from this study will have an immense use for seed testing stations, seed technologists, breeders as well as seed companies and seed traders. In this context the study was undertaken with the following objectives:

1. To standardize preconditioning of seeds for tetrazolium staining,
2. To study the effects of different concentrations of tetrazolium solutions and incubation temperatures on the staining pattern and
3. To compare the efficacy of tetrazolium viability results with standard germination and field emergence and also to compare its economy over standard germination test.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

In this chapter an effort is being made to present the views and suggestions made by various workers on the methodology and conduct of tetrazolium test and its application as a viability test for different seeds, its drawbacks as well as advantages over the other viability tests. There are also some reviews regarding standard germination tests and its reliability to determine the field emergence potentials of a given crop seed.

Moore (1970) opined that the tetrazolium test can be a powerful tool in seed quality control schemes and seed quality evaluation, if used properly.

Sevilla (1987) reported that the literature on tetrazolium test was insufficient and most of the available literature was in foreign language inspite of the fact that it has wider application among the seed testing laboratories around the world. Since this test has not been recommended for routine testing of seed viability of pulses, the informations regarding tetrazolium viability test in pulses are hardly available and that is the reason much of the reviews cited here are on the other crop species.

2.1 Tetrazolium test for seed viability studies

Lakon (1942), the German scientist was the first person to report the use of 2,3,5-Triphenyl tetrazolium chloride for testing the viability of seeds. Later several workers have successfully used it for the topographical determination of seed viability (Cotrell, 1947, Yanagisawa and Asakawa, 1953; Mukherji, 1956; Roistacher and Nauer, 1961). This method has gained popularity on account of its simplicity, rapidity in application and the manifestations can be clearly recorded and interpreted. Standard procedures for different crop species have beenworked out in various parts of the world (Anon, 1966; Gopal and Thapliyal, 1969; Grabe, 1970).

Costa and Bemis (1972) studied seed viability of xerophytic gourd *Cucurbita foetidissima* HBK using tetrazolium test and it recorded 90 per cent on 38th day after pollination, whereas the laboratory germination test gave only 29 per cent, indicating dormancy.

Agrawal *et al*. (1973) used tetrazolium test to predict the germinability in maize, wheat and paddy seeds.

Prakophun (1981) used TZ test for evaluating the viability of soybean seeds which were harvested using different threshers.

Harty *et al*. (1983) stored the seed lots of *Panicum maximum* cv.. makueni Gatton and Patri Green panic for three years at three different temperatures, and assessed the viability of seeds using TZ test. There was a close correlation between the viability of seeds at the beginning of storage as determined by TZ test and mean maximum germination.

Navotna (1984) tried tetrazolium test on 32 spp. of medicinal plants from 13 families. The investigations showed that tetrazolium method may be applied to the seeds of all species of medicinal plants which were being examined.

Moore (1985) described the preparation and evaluation of tetrazolium test methodologies for 177 species of trees and shrubs, 98 agricultural species, 335 flower species, 53 vegetable species, and 22 herb and medicinal plant species.

Dias and Silva (1986) found tetrazolium test as a reliable method for testing the viability of *Coffea arabica* seeds.

Mohamed *et al*. (1988) studied the viability of seeds in two egg plant species viz., *Solanum melongena* var. insanum and *Solanum aethiopicum*, in which tetrazolium test successfully descriminated between dead and dormant seed lots.

Rennie and Gorey (1988) estimated the germination potential of winter wheat and winter Barley seeds using tetrazolium tests.

Figueiredo (1989) determined the germination of Jojoba [*Simmondsia chinenesis* (Link) Schneider] seeds successfully by using TZ test.

Sader *et al*. (1991) studied the influence of size and processing operations on mechanical injury in groundnut using tetrazolium test. Results showed a decrease in the percentage viability after threshing or grading, but seed viability was unaffected by seed size differences.

Alliprandini *et al*. (1992) conducted viability test in dormant seed lots of Andropogon grass (*Andropogon gayanus* Kunth) which indicated 89 per cent of the caryopses were viable.

Neto *et al*. (1993) carried out vigour and TZ viability test in soybean, whose seed quality was affected by shrivelling due to heat and drought stresses during seed filling. The test indicated a significant reduction in seed quality as the level of shrivelling increased.

2.2 Mechanism of tetrazolium reaction

Lakon (1942) recognised that all living tissues which respire, are capable of reducing a colourless chemical, 2,3,5-triphenyl tetrazolium chloride or bromide into a red compound formazan by the enzyme dehydrogenases, which catalyse hydrogen ion transfer reactions. Formazan being non-diffusable, stains the living tissues into red. Thus the living part of a viable seed should be stained red when incubated in TZ solution.

2.3 Preconditioning of seeds

Preconditioning of seed is done to allow complete hydration of all tissues, to prevent damage to cotyledons and embryonic axis while cutting seeds, to initiate and activate the germination process and to have proper penetration of tetrazolium solution.

Lovato and Amaducci (1964) conducted tetrazolium viability test in onion and leek seeds wherein the seeds were soaked for 6 and 15 hours in distilled water at room temperature. Both the soaking periods gave good staining which were correlated well with standard germination test results.

Grabe (1970) reported that small seeded crops and seeds with a permeable seed coat can be moistened by direct soaking without any adverse effects of soaking injury. The moistening time can be considerably reduced by putting the seeds at a slightly higher temperature, up to 40 ^{o}C during imbibition. For large seeded legumes, slow imbibition between moist blotters overnight has been recommended. Seed coats of those small seeded legumes which are permeable to tetrazolium can be directly immersed in TZ solution without any preparation. Seeds having red coloured fungicide treatment should be washed before conditioning. Thus the choice of conditioning method depends on the speed and accuracy required and the characteristics of seeds.

Agrawal *et al*. (1973) predicted the germinability in maize, wheat and paddy seeds on the basis of tetrazolium test. For preconditioning, they soaked maize and paddy seeds in water for about 15 and 30 hours respectively, whereas wheat seeds were soaked in a film of water in a petridish for about 15 hours. Later they bisected the seeds and transferred them to 0.5 per cent TZ solution.

Agrawal and Kaur (1975) during standardization of TZ test for Ragi (*Eleusine coracana* L.), soaked the seeds in water at low temperature of about 5 °C for 18 hours before bisecting the seeds.

Gupta and Raturi (1975) filed-off a fragment of testa in case of three *Albizia* spp. and two *Cassia* spp.in order to prepare them for imbibing water during the conduct of tetrazolium viability study. Afterwards these were soaked in water for 16 hours and decoated before putting them in TZ solution of 0.5 per cent concentration.

Yaklich and Kulik (1979) tried to diagnose the causes of seed weakness in soybean using tetrazolium test, wherein the seeds were allowed to imbibe water in rolled paper towel and incubated at 25 °C for overnight. Later these seeds were placed in water for completing the hydration process prior to preparation for staining.

Pasha and Das (1982) suggested optimum soaking period of four hours for soybean seeds in distilled water while conducting TZ test.

Ellis et al. (1985) reported that to moisten the seeds quickly, preconditioning is often carried out at 30 °C. Longer periods of soaking at lower temperatures or shorter periods of soaking at higher temperatures however, may be equally satisfactory and when very dry seeds are soaked, it may result in imbibition injury and hence, there is a need of humidification treatment prior to conditioning. They suggested 18 hours of imbibition or soaking for *Cicer arientinum* L., slow imbibition up to 18 to 24 hours and then soaking in water for two to three hours in case of *Phaseolus* spp. and for *vigna* spp. 22 hours soaking has to be followed for preconditioning as recommended by the International Seed Testing Association.

Costa and Filho (1994) studied the possibility of reducing the pre-conditioning period before the TZ staining process by using nine soybean [*Glycine max* (L.) Merr.] lots (3 cultivars) which were imbibed for four periods viz., 4,6, 8 and 10 hours at two temperaturs (35 and 42 °C). The standard procedure for soybean seeds (25 °C, for 16 hours) was used as the control. It was concluded that the preconditioning period could be reduced for determining viability and vigour potentials for soybeans. Imbibition at

42 °C for 8 and 10 hours and eventually, for six hours resulted in adequate staining of the seed structures.

2.4 **Preparation for staining**

Preparation of the seed is the step in which the embryo and other essential structures of the seed are exposed for better penetration of TZ solution and staining.

Anon (1966) recommended preparation methods in 28 tree species seeds. Preparation methods for TZ testing like cutting of 1/3rd of the fruit at the broad end opposite to the radicle, after the fruits were soaked in water for 18 to 20 hours in case of Carpinus spp. and removal of seed coat by means of dissecting needle followed by clipping-off the 0.5 mm each of the radicle tip end of the cotyledons opposite to the radicle in case of Acer spp. and similar methods of preparation have been recommended for 26 other species (Anon., 1966).

Grabe (1970) gave detailed procedure of preparation for staining in case of large seeded and small seeded grasses, large and small seeded legumes and other dicots. He also opined that no preparation is needed for cowpea, chickpea, soybean and peas other than removal of seed coat before staining.

Agrawal et al. (1973) while conducting TZ test in maize, wheat and paddy, bisected one hundred seeds longitudinally so as to expose the embryo ofter preconditioning. During bisecting the seeds, one half of each seed was discarded and other half kept in small amount of water before transferring to 0.5 per cent of freshly prepared TZ solution.

Gowrishanker and Ranganath (1974) bisected the seeds of Amla (Phyllanthus emblica L.) and Guava (Psidium guajava L.) into equal halves longitudinally, whereas in Ber (Zizyphus mauritiana L.) the hard seed coat was first removed followed by bisecting the seeds into two halves longitudinally before putting in one per cent tetrazolium solution.

Agrawal and Kaur (1975) soaked Ragi (Eleusine coracana L.) seeds and bisected them in such a way that the embryo of each seed was divided into identical halves before soaking in 0.5 per cent TZ solution.

Yaklich and Kulik (1979) bisected the imbibed soybean seeds through the embryonic axis by using a razor blade and removed the seed coat before treating with TZ solution.

Macchia (1981) conducted viability tests in several species of wheat, barley and grass seeds using tetrazolium salt. Here the preparation was made by bisecting the seeds rather than by embryo extraction.

Pasha and Das (1982) pealed off the seed coat in soybean after complete imbibition of seeds during the conduct of TZ test for viability asesement.

Ellis *et al.*(1985) suggested puncturing of the seed coat or cutting at the distal end opposite to the embryo for small seeded grasses and bisecting longitudinally through the embryo in case of large seeded grasses, cereals and members of compositae.

Bebele and Kandya (1986) bisected the imbibed seeds of *Lagerstroemia parviflora* (Roxb) longitudinally before putting in 0.01 per cent TZ solution for the viability study.

Patil and Dadlani (1993) recommended removal of seed coat using forceps or needles or razor blades, etc. The thin -membrane adhering to the cotyledone may be removed by a sliding motion after an additional soaking of 30 minutes, especially for dicots with hard seed coats.

Schatral and Fox (1994) assessed the seed viability of six species of *Hibbertia* using TZ test. After one day imbibition in distilled water, the seed coat was removed and the endosperm was cut near the microphyle with a sterile scalpel. This technique exposed the minute embryo (0.1 mm). No attempt was made to remove the embryo because of its small size. The endosperm sections were placed on moist filter paper in petri-dishes before soaking in one per cent 2,3,5-Triphenyl tetrazolium chloride solution.

2.5 Preparation of tetrazolium solution

Different concentrations of tetrazolium salt solutions have been used with comparable results. Conveniently stock solutions of 1.0 and 0.1 per cent may be maintained. One per cent solution used for seeds that are not bisected through the embryo, while 0.1 per cent used for seeds in which the embryo is bisected. Other intermediary concentrations like 0.2, 0.5 per cent etc. can also be used (Grabe, 1970; Ellis _et al_., 1985).

To preapare one per cent solution, 1 g TZ salt has to be dissolved in 100 ml distilled water, or tap water with neutral pH. To prepare 0.5 per cent solution, one part of one per cent solution has to be mixed with one part water or 1 g TZ salt dissolved in 200 ml water. To prepare 0.1 per cent TZ solution, one part of one per cent solution has to be mixed with nine parts of water or 1 g tetrazolium salt dissolved in one litre water (Grabe, 1970; Patil and Dadlani, 1993).

After the preparation of TZ salt solution, it should be stored in amber coloured bottles and if it is stored in dark at low temperature of 3 ^{o}C, it can be preserved for months together (Ellis _et al_., 1985).

2.5.1 pH of the tetrazolium solution

Lindenbein (1965) suggested that the acidity of TZ solution should be kept neutral (pH 7.0), which excludes the possibility of other compounds in the cell acting on the reducing agent.

If the pH of the distilled water is not within the range of 6.5 to 7.0, the tetrazolium salt should be dissolved in a buffer solution. The buffersolution can be prepared as follows.(Anon.,1966).

Solution 1: 9.078 g of KH_2PO_4 dissolved in one litre of water.

Solution 2: 11.876 g of $Na_2HPO_4.2H_2O$ dissolved in one litre of water.

Later, 400 ml of solution 1 and 600 ml of solution 2 are to be mixed together. 10 g of TZ salt dissolved in one litre of the buffer solution gives 1.0 per cent TZ solution with 7.0 pH (Anon., 1966).

Gupta and Raturi (1975) prepared 0.5 per cent of TZ salt solution in double distilled water of approximately 6.5 to 7.0 pH for testing the seed viability of six Indian tree species.

2.5.2 Role of tetrazolium salts on the accuracy of test results

Steiner and Kaysan (1984) tested the purity of different 2,3,5-Triphenyl tetrazolium chloride salts based on the melting point, decomposition point, absorption coefficient and depression of pH in aqueous solutions in case of 14 commercially available 2,3,5-Triphenyl tetrazolium chloride products. No product was pure and the wider differences in purity especially with regard to their effect on the pH of aqueous solution, which would affect their usefulness in seed testing.

Steiner and Werthe (1984) tested the differences in viability value using different 2,3,5-Triphenyl tetrazolium chloride products. The viability of Lupinus perennis and Rye seeds were tested by incubating in unbuffered solutions of each of 14 commercially available 2,3,5-Triphenyl tetrazolium chloride products was in the range 7 to 21 and 0 to 80 per cent respectively, compared with 43 to 53 and 78 to 86 per cent in the same solutions phosphate buffered to pH 7.0 and was highly correlated to solution pH.

2.6 Staining

During tetrazolium test, staining of seed tissues is influenced by incubation temperature, concentration of TZ solution, duration of incubation and seed characteristics such as age group of the seed.

Anon (1966) mentioned standard temperature and duration of soaking in tetrazolium solution for the seed of 28 tree species, which varied from 18 to 48 hours of soaking in one per cent TZ solution having neutral pH. Further, a much faster reaction can be obtained in Pinus cembra and Pinus coulteri, if the seeds are treated for six hours in a solution of TZ in vacuum at 45 ^{o}C and later keeping in the solution 16 hours longer at 30 ^{o}C.

Grabe (1970) opined that the accuracy of TZ test does not get effected if it is conducted in the temperatures between 20 ^{o}C and 45 ^{o}C; but staining proceeds faster at high temperatures. For shorter test periods, staining can be done by placing the materials at 30 ^{o}C in seed germinators. Temperatures above 45 ^{o}C should not be used and as a general rule of thumb, staining will take place twice as fast at 30 ^{o}C as at 20 ^{o}C and twice as fast at 40 ^{o}C as at 30 ^{o}C. Further, the test may be performed in subdued light or in the dark for staining; however, light has little effect on the tetrazolium test. He also suggested the use of 1.0 per cent TZ solution with staining time of three to four hours at 35 ^{o}C for chickpea, cowpea, peas and soybeans.

Agrawal et al. (1973), while conducting viability test in case of maize, wheat and paddy seeds, soaked the bisected seeds in TZ solution of 0.5 per cent concentration, after preconditioning. Seeds soaked in TZ solution were kept at 30 ^{o}C in dark for about three to four hours. The TZ solution was then discarded and seeds were rinsed two to three times in water and evaluated for percentage of viable seeds.

Gupta and Raturi (1975) assessed the seed viability of six Indian tree species. Here, soon after the preparation, the seeds were placed in 0.5 per cent TZ solution in petridishes covered with black paper and kept at 30 ^{o}C for 24 hours.

Unalcin (1979) predicted sunflower seed viability using TZ salt. Use of 1.0 per cent TZ solution with staining period of 1.0 to 1.5 hours at 35 °C was found to stain the seeds satisfactorily.

Jethani (1982) studied the viability of coriander (*Coriandrum sativum* L.) seeds by soaking the halved seeds in TZ solution of 0.5 per cent concentration for three hours at 40 °C.

Pasha and Das (1982) determined seed viability of five cultivars of soybean. After preconditioning and preparation, seeds were soaked in 0.5 per cent TZ solution in petriplates covered with black paper and kept for six hours for complete staining.

Ellis *et al*. (1985) suggested TZ staining treatment for 6 to 24 hours using 1.0 per cent TZ solution at 30 °C for *Cicer arietinum* L., and 0.5 to 1.0 per cent solution with 6 to 24 hours soaking period at 30 °C for *Phaseolus* spp. and 0.5 to 1.0 per cent TZ solution with 16 to 24 hours staining period at 30 °C for *Vigna* spp. after preconditioning and removal of seed coat as recommended by the International Seed Testing Association.

Haris *et al*. (1985) estimated the viability of *Hevea* seeds by using TZ test. A comparison of soaking duration and temperatures for testing the viability using 1.0 per cent TZ solution showed that four hour soaking at 40 ^{o}C produced uniform red staining of cotyledons and embryonic axes.

Vorovenci and Cseresnyes (1985) presented an improved method of analysing Lucerne seed viability wherein the seeds were stained by soaking 16 hours initially and five hours in 1.0 per cent TZ solution at 30 ^{o}C in dark gave best results which could be comparable with standard germination results especially in seeds with higher germination.

Yadav _et al_. (1986) in their study on quick test of viability and vigour of _Chloroxylon sweitinia_ D.C., soaked the bisected seeds in 0.1 tetrazolium solution for 20 hours. Seed were preconditioned before bisecting.

Lotito (1989) tested seed viability of okra (_Abelmoschus esculentus_ (L.) Moench.). Seed coat was removed from the imbibed seeds and the embryos were immersed in 1.0 per cent TZ solution prepared in 6.0 to 8.0 pH phosphate buffer and incubated at 35 ^{o}C for two hours in dark.

Das and Senmandi (1992) assessed tetrazolium solution stainability of different viability wheat seeds by immersing the bisected seeds in a freshly prepared solution of 0.2 per cent concentration and incubated for 20 minutes at 30 ± 1 ^{o}C.

Patil and Dadlani (1993) given tetrazolium testing methods for some important Indian crop species, in which they mentioned the staining time of 6 to 24 hours for chickpea and pigeonpea; 18 to 24 hours for cowpea and greengram at 35 °C, using 1.0 per cent tetrazolium chloride solution.

Schatral and Fox (1994) assessed the viability of Hibbertia seeds using TZ salt. After preconditioning, the embryo of the seed exposed and the endosperm sections along with the embryo were placed on moist filter paper in petri dishes, then soaked with a 1.0 per cent sterilized 2,3,5-Triphenyl tetrazolium chloride solution and left for at least six hours and a maximum of 24 hours in dark conditions at room temperature (21 to 23 °C). Viable embryos were red or pink while the non-viable embryos either remained unstained or showed large unstained areas.

2.7. Evaluation of stained seeds

Evaluation of topographical staining pattern is the most critical and tricky step in TZ test, for which there is a need of experience (Lakon, 1942).

At the time of evaluation, one should note that bright cherry colour indicates living tissue and pink colour is when the solution is not penetrated well or if bacteria might be grown on the tissues which reduce the tetrazolium salt. Dark colour is not the absolute indication of physiological activity of the cell (Lindenbein, 1965).

Anon (1966) made four viable classes in Acer, Carpinus, Corylus, Cotoneaster, Rosa, Crataegus, Sorbus, Fagus, Pyrus, Malus and Prunus. spp. and two classes in case of Tilia and Fraxinus spp. based on topographical patterns of staining.

Grabe (1970) suggested the application of Lactophenol clearing solution to the tetrazolium treated seeds to facilitate easy evaluation of small seeded grasses and also recommended the use of stereoscopic microscope for evaluation. Accurateinterpretation of TZ test depends on sound knowledge of seed and seedling structures, seed germination, understanding the mechanisms of reactions leading to staining and combining interpretations of staining patterns with other visible aspects of seed quality as well as experience in making comparative germination tests. He also presented 15 topographical staining patterns for legume seed viability evaluation.

Moore (1970) opined that, differences can be obtained in staining patterns due to mechanical injuries, water damage, ageing, freezing, heat injury, diseases, insects and calcium deficiency.

Gupta and Raturi (1975) presented 10 topographical staining patterns in Albizia lebbeck (L.) Benth, 12 in Albizia procera (Roxb.) Benth, 15 in cases of Albizia chinensis (Osbeck) Merr. and 12 in Cassia fistula Linn., 7 and 6 categories respectively in Cassia nodosa Ham., and Pinus roxburghii Sargent.

Yaklich and Kulik (1979) evaluated soybean seeds as viable, non-viable and deteriorating tissues by using tetrazolium staining. Germinable seeds were rated from one to five and non-germinable ones from six to eight. Seed rated one if all its tissues appeared to be viable. Seeds with less than 100 per cent viable tissues or showing critical injuries were assigned higher values.

Toruan (1982) tested the viability of rubber (*Hevea brasiliensis* M.A.) seeds, where the seeds were classified as viable when the embryonic axis and cotyledons stained red and seeds which have deteriorated did not stain evenly.

Yadav *et al*. (1986) tested the viability of seeds in *Chloroxylon sweitinia* D.C. by using TZ test and made seven topographical categories of staining for the assessment of viability.

Don *et al*. (1990) reported the difficulties occurred in the viability assessment of seven samples of spring barley seeds which had received pre-harvest glyphosate treatment. The tests were conducted in seven stations of the International Seed Testing Association. Seeds which have been killed by glyphosate can produce a tetrazolium staining pattern that enables them to be classified as viable ones.

Das and Senmandi (1992) made six categories of embryo staining in fresh and artificially aged wheat seeds and 10 categories in case of naturally aged seed stocks during TZ viability studies.

Patil and Dadlani (1993) given three evaluation codes each for chickpea, cowpea, greengram and two categories or codes in case of pigeonpea based on topographical staining patterns for the viability study.

2.8 Relationship between tetrazolium test of seed viability and standard germination test

Grabe (1970) reported that discrepancies between germination test results and tetrazolium test results include sampling differences, improper tetrazolium testing techniques, dormant and hard seeds, seed borne organisms and chemical injury. If tetrazolium test is done by an experienced person, it can be used for supplementing germination tets.

Laszlo (1972) reported a more rapid decrease in the germination capacity of pea seeds compared to dehydrogenase activity, but a close correlation was found between tetrazolium test results and germination capacity.

Yaklich and Kulik (1979) conducted laboratory tests for standard germination, seedling vigour classification, seedling length and tetrazolium staining in 144 and 81 seed lots of soybean in 1975 and 1976 respectively. Most of the laboratory test measurements were significantly correlated with field emergence.

In case of Achillea millefolium, Artemisia tridentata and some other shrubs and forbs, TZ test did not distinguished between dormant and non-dormant seeds (Weber and Wiesner, 1980).

Bumarlong (1981) tested the viability of seeds of four forest tree species, viz., Sweitenia macrophylla King., Samanea saman (Jacq. Merr.) Leucaena leucocephala (Lam.) de Wit. cf. cv K8., Albizia procera (Roxb). Benth, using 2,3,5-Triphenyl tetrazolium chloride, which was done by comparing all TZ treatments with actual germination tests. Statistical analysis of percent germination or stained seeds showed highly significant differences among the treatments applied to Sweitenia macrophylla King, Leucaena leucocephala (Lam.) and Samanea saman tree seeds. A significant difference among the treatment was obtained for Albizia procera (Roxb). Benth.

Cseresnyes et al. (1982) assessed the germination capacity of barley and wheat seeds wherein a good correlation was found between germination capacity as assessed by TZ test and standard germination test.

In case of mechanically damaged soybean seeds, TZ test indicated a significantly higher percentage laboratory germination than the standard germination test . Thus TZ test was not sensitive in detecting recently induced mechanical damage. The TZ test over - estimated average field emergence from zero to 20 per cent during 1975, whereas the estimated average field emergence was within ± 8 per cent. In the next year standard germination test estimated average field emergence within ±12 per cent but TZ test generally over estimated average field emergence from zero to 18 per cent (Mason et al., 1982).

Steiner and Fuchs (1982) determined the seed viability of wheat by using TZ test which was 96 per cent whereas in germination tests, the fungicide treated seeds gave 94 to 96 per cent normal seedlings under all the conditions used but with untreated seeds, the percentage of normal seedling was 81 per cent on paper at 20 ^{o}C, 77 and 70 per cent in sand and soil respectively at 15 ^{o}C.

Umamaheswar and Mahadevappa (1983) tested the viability of freshly harvested and three year old paddy seeds using TZ salt along with standard germination test. They found good correlation between these two tests.

Solanki et al. (1984) assessed the viability of 28 lentil cv. using an aqueous solution of tetrazolium solution and standard germination test at 30 ^{o}C. Seed germination varied from 35.0 to 81.3 per cent among the cultivars and it was concluded that the TZ test although comparable and complementary to germination as a measure of seed viability did not assess the percentage of hard seeds.

Ellis <u>et al</u>. (1985) suggested not to consider TZ test as an absolute test of seed viability. There is a need to make comparison for each species, of the staining patterns observed in tetrazolium tests with the results of germination test on the same accessions, where the results of the latter have not been confounded with either dormancy or poor seed quality; that is where all viable seeds are known to have germinated. Further, hard seededness or delayed imbibition has lead to germination test underestimating accession viability, TZ tests will suffer from the same deficiency.

Rytko <u>et al</u>. (1985) stated that germination capacity and viability of wheat, barley and rye seeds were one to two per cent higher when estimated by TZ test than by the filter paper test. The difference was most marked in the seeds of low sowing value. Use of full staining by TZ as a test of viability improved correlation with field emergence.

Coster (1988) conducted two experiments, one in 1986 with 80 samples of winter wheat, winter barley and spring wheat, and in 1987 with 150 samples of winter wheat, spring barley and spring wheat, wherein the TZ test and sequential sampling were comparable with standard germination test results.

Das and Senmandi (1992) reported that in case of wheat, comparison of the mean germination percentage on the basis of TZ staining and mean germination test were found to have a good correlation, both in naturally aged and artificially aged wheat seeds.

2.9 Relationship between standard germination and field emergence test results

Due to favourable conditions created in laboratories for the germination tests, the percentage germination recorded in laboratories may exceed field emergence results for the same seed lot tested, if the seeds are planted under unfavourable field conditions. Erickson and Porter (1938) conducted field emergence tests in Alfalfa and Red clovers wherein the emergence of seedlings was considerably less than laboratory germination. Further, the higher germinating lots responded best in the field.

The total germination value as determined by standard germination test may fail to predict field stand with sufficient accuracy, usually because of their failure to detect weaknesses which may be present in the seed (Isley, 1958; Delouche and Caldwell, 1960).

In a viability study of soybean, a highly significant correlation was obtained between the laboratory germination and field emergence test results (Woodstock *et al.*, 1970; Agrawal and Singh, 1975).

Perry (1977), Egli and Tekrony (1979) opined that if standard germination test is conducted under controlled conditions for seed germination, when field conditions at planting are near optimum, the standard germination test results correlated very well with field emergence.

Mason *et al*. (1982) reported that the differences in the quality of soybean seeds caused variable germination performance both in the laboratory and in the field. Germination test accuracy was measured by analysing the difference between field emergence and germination test results.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The material and methods adopted during the present investigation have been presented in this chapter.

The detailed procedures followed for standardization of preconditioning in six pulse crop seeds, standardization of TZ staining periods at different temperatures along with different concentrations of TZ solutions and evaluation of topographical staining patterns as well as the test procedures followed for standard germination and field emergence have also mentioned in this chapter. The laboratory tests were conducted in the laboratory, Department of Seed Technology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bangalore - 560 065 and the field emergence test was conducted at Seed Testing Research Unit, National Seed Project, Gandhi Krishi Vignana Kendra, Bangalore - 560 065.

3.1 Experimental materials

3.1.1 Seeds

Seeds of six pulse crops viz., cowpea (Cv. C-152), greengram (Local variety), bengalgram (local variety), horsegram (local varity) and redgram (Cv., Hyderabad 3C) and blackgram (Cv. T9) have been collected from different sources in Bangalore for the present study. The seed lots were of six to eight months old. The seeds have been precleaned and tested for initial moisture content, germination and seed health (Appendix 1). The seed lots which had higher moisture content than the storage limits were dried to safe moisture level and stored in polythene covers in ambient conditions for the future studies.

3.1.2 Tetrazolium salt

During the course of investigation, two brands of TZ salt have been used; one supplied by LOBA CHEMIE PVT LTD., BOMBAY and the other by S.d. FINE CHEM LTD. The former brand was used for testing cowpea, greengram, bengalgram and horsegram whereas the latter was used for redgram and blackgram.

The use of different TZ salt brands for a given crop seed for any comparative tests were avoided to exclude the variances caused by the salts (Steiner and Kaysan, 1984; Steiner and Werthe, 1984).

3.2 Initial seed quality

Tests of seed moisture content, seed viability and seed health were conducted before storing the seeds have been included here.

3.2.1 Seed moisture content

The moisture content of pulse crop seeds was determined as per the International Seed Testing Association rules (Anon., 1985) by using high constant temperature oven method. Five grams of seed after grinding was taken in three replications and kept at constant temperature of $130 \pm 2\,^{o}C$ for 2 hours and at the end of drying period, the cups were cooled in a desiccator using silica gel for 30-45 minutes. The moisture content was calculated using the formula (Appendix I).

$$M = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where, M = Moisture content on wet basis (in %)

W_1 = Weight of empty dish (g)

W_2 = Weight of dish with seed before drying (g)

W_3 = Weight of dish with seed after drying (g)

3.2.2 Standard germination test

The standard germination tests for all the six pulse crop seeds were conducted by following International Seed Testing Association Rules (Anon., 1985). Between paper (BP) method was followed in all the tests and constant temperature of 25 ^{o}C was maintained in the incubator throughout the test period. First and final counts were taken and per cent germination was calculated on the basis of normal seedlings (Anon., 1979, 1985). Along with the normal and abnormal seedlings, the fresh ungerminated, dead/decayed, hard seeds and diseased seeds were also noted (Appendix 1).

3.2.3 Seed health test

Seed health test was conducted to know the presence of seed borne pathogens by following International Seed Testing

Association Rules (Anon., 1985). Eight replications were used with 50 seeds in each replication. Top of the paper (TP) method was followed, by using moistened blotters. The petridishes were incubated at constant temperature of 20 °C in seed germinator. The plates were observed for the development of microbial colonies (Appendix 1).

3.3 Preparation of TZ solution

Different concentrations viz., 0.10, 0.25, 0.50, 0.75 and 1.00 per cent were prepared using tap water whose pH before and after the preparation of TZ solution was found to be in the range of 6.8 to 6.9.

To prepare 1.0 per cent salt solution, 1 g TZ salt was dissolved in a small quantity of tap water and then the volume was made up to 100 ml. One per cent TZ salt solution was used as stock solution to prepare other concentrations as in Appendix 3; as prescribed by Grabe (1970) and Ellis et al. (1985).

Every time, the freshly used TZ solution was used for the study. The TZ solutions (viz., 0.10%, 0.25%, 0.50%, 0.75% and 1.00%) were stored in amber coloured bottles in dark, at room temperature.

3.4 Equipments

1. Test tubes, petridishes, beakers, pipetts, measuring jars, razor blades, needles and forceps.
2. Incubator.

3. Amber coloured bottles.

4. Dissection microscope and hand lens.

5. Balance with 0.001 g accuracy.

6. pH papers.

3.5 Standardization of pre-conditioning period and preparation for TZ treatment

Hundred pure seeds of a given pulse crop were put in a beaker containing water. Seed to water ratio of 1:3 v/v was maintained. At different time intervals, the easiness with which seed coat could be pealed-off has been checked. The optimum time required for almost all the seeds of a given lot to imbibe water completely has been recorded.

There was no special preparation method prescribed for pulse crop seeds prior to TZ staining. Soon after preconditioning, the seed coats of these seeds were pealed-off gently using finger nails. Care has been taken to avoid any damage to the radicle or any other parts of the embryos (Ellis et al., 1985).

3.6 Standardization of treatment duration

Fifty seeds were soaked in water for a specific period based on preliminary soaking studies. Later the seed coats were removed and the seeds were placed in TZ solution having the concentration of 0.1 per cent and incubated at 25 °C in an incubator. Similar procedures were followed for all other TZ solution concentrations viz., 0.25, 0.50, 0.75 and 1.00 per cent. The above procedures were repeated in different temperatures viz., 30 ^{o}C, 35 ^{o}C and 40 ^{o}C in an incubator. The optimum colour development for satisfactory evaluation of seeds was the prime objective, hence the treated seeds were observed frequently for optimum colour development and the reaction time taken for the development of colour was recorded separately for different temperature and TZ solution concentrations.

3.7 Comparative standard germination, field emergence and TZ viability tests

After standardizing the optimum pre-conditioning time and period of TZ staining reaction at different temperatures and TZ concentration treatments, the comparative studies wereconducted with the prime objective of standardizing TZ evaluation - patterns and comparing the seed viability as asssessed by TZ test with that of standard laboratory germination as well as field emergence test results.

3.7.1 Number of seeds taken for the study

In total 400 pure seeds were taken in each pulse crop for standard laboratory germination test. Totally 8 replicates were taken with 50 seeds in each replication (Anon., 1985).

For the field emergence study, 200 pure seeds were taken and sown in cement pots of size 2.5' x 1.5'. Eight replicates of 25 seeds each had been used for the study.

For the TZ test, in each temperature and TZ solution combinations, 200 pure seeds were tested with eight replicates of 25 seeds each was used (Grabe, 1970; Moore, 1985).

3.7.2 Standard germination test

The standard laboratory germination test was conducted to compare the efficacy of TZ test results. The test procedures were same as that described in 3.2.2 part of this chapter.

3.7.3 Field emergence test

Field emergence test was conducted at Seed Testing Research Unit, National Seed Project, Gandhi Krishi Vignana Kendra, Bangalore-560 065, by using cement pots of size 2.5' x 1.5', filled with soil. Eight replicates; each with 25 seeds were sown in each of the crop. Field emergence was recorded seven days after sowing.

3.7.4 Tetrazolium test

Steps

1. Two hundred pure seeds were soaked in water. Seed to water ratio of 1:3 v/v was maintained and kept for pre-conditioning for the period as standarized earlier (Table 1).

2. Seed coats were carefully pealed off by hand and seeds were put in test tubes containing 0.1 per cent TZ solution with eight replications.

3. The above test tubes with the materials were incubated at 25 ^{o}C. Complete immersion of all the seeds by the solution was ensured before keeping for incubation.

4. After the completion of TZ staining (Table 2) period, the stained seeds from all the replicates were taken in petridishes separately and washed in water three to four times to remove the traces of TZ solution.

5. To avoid drying, the treated seeds after washing were kept immersed in a thin film of water in a petridish until evaluation.

6. Stained seeds were evaluated based on the topographical staining patterns.

The above steps have been recommended by Grabe (1970) and Ellis et al. (1985).

The above procedure was repeated for other TZ solution concentrations viz., 0.10, 0.25, 0.50, 0.75 and 1.00 per cent as well as for other incubation temperatures viz., 30, 35 and 40 ^{o}C for all the six pulse crop seeds taken for the present investigation.

3.8 Comparison of TZ test economy with that of standard germination test

The following cost calculations were made to compare the economy of TZ test over standard germination test.

Number of seeds taken for TZ test was 200 (Grabe, 1970) and for standard germination test it was 400 (Anon., 1985).

TZ test	Standard germination test
1. Cost of TZ salt needed to prepare TZ solution to test 200 seeds	1. Cost of germination paper to test 400 seeds in four replicates of 100 seeds each
2. Labour time needed (man hours) for the TZ test	2. Cost of wax sheets
3. Total time needed for TZ testing which includes the preconditioning time, preparation time, staining period as well as the time needed for evaluation.	3. Labour time needed (in man hours) for conducting standard germination test.
	4. Total test period (in days).

3.9 Test design

To compare the efficacy of tetrazolium test with that of standard germination and field emergence tests, completely Randomized Design was used. The percentage values obtained in each staining pattern classes were added successively until the value nearest to the standard germination percentage of the cultivar was obtained (Gupta and Raturi, 1975). These categories were expected to form viable categories. To arrive at the correct conclusion, a 't' test was applied to various category combinations. Combinations exhibiting the lowest 't' values were taken to be the most feasible. The total percentage values of the categories of these combinations were compared with the actual germination percentage (Pasha and Das, 1982).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the present study on the comparative efficacy of tetrazolium test for evaluating the seed viability of major pulse crops are presented under the following headings:

1. Time needed for preconditioning of seeds.
2. Time required for TZ staining at different incubation temperatures and different TZ solution dilution.
3. Standard germination and field emergence test results.
4. Tetrazolium test results.
5. Comparative economy of TZ test over standard germination test.

4.1 Time needed for preconditioning of seeds

The optimum soaking time needed for the six pulse crop seeds at room temperature is presented in Table 1 and Fig. 1.cowpea has taken 6 h soaking time. grengram, horsegram and redgram have taken seven hours whereas blackgram and bengalgram have taken 8 hours for preconditioning at room temperature.

4.2 Time required for TZ staining at different incubation temperatures and different TZ solution dilutions

The optimum incubation time for the TZ reaction to occur at different incubation temperatures, viz., 25, 30, 35 and 40 ^{o}C and five TZ solution concentrations viz., 0.10, 0.25, 0.50, 0.75 and 1.00 per cent have been presented in Table 2 and Fig. 2a,b and c.

Table 1: Time required for preconditioning* of different pulse crop seeds for TZ test

Crop	Botanical name	Variety	No. of seed pealed** off								Soaking time (hours)
			1	2	3	4	5	6	7	8	
Cowpea	Vigna unguiculata (L.) Walp	C-152	00	06	30	59	89	100	100	100	6.00
Greengram	Vigna radiata (L.) Wilczek	Local	00	15	43	65	83	92	100	100	7.00
Horsegram	Dolichos biflorus L.	Local	00	08	29	72	88	96	100	100	7.00
Blackgram	Vigna mungo (L.) Hepper	T-9	00	00	15	39	55	72	91	100	8.00
Bengalgram	Cicer arietinum L.	Local	00	04	22	38	51	69	88	100	8.00
Redgram	Cajanus cajan (L.) Mill sp.	Hyd.3C	00	10	32	53	77	93	100	100	7.00

* At room temperature (25 ± 1^{o} C)
** Average value of 2 replications (50 seeds x 2 replications)

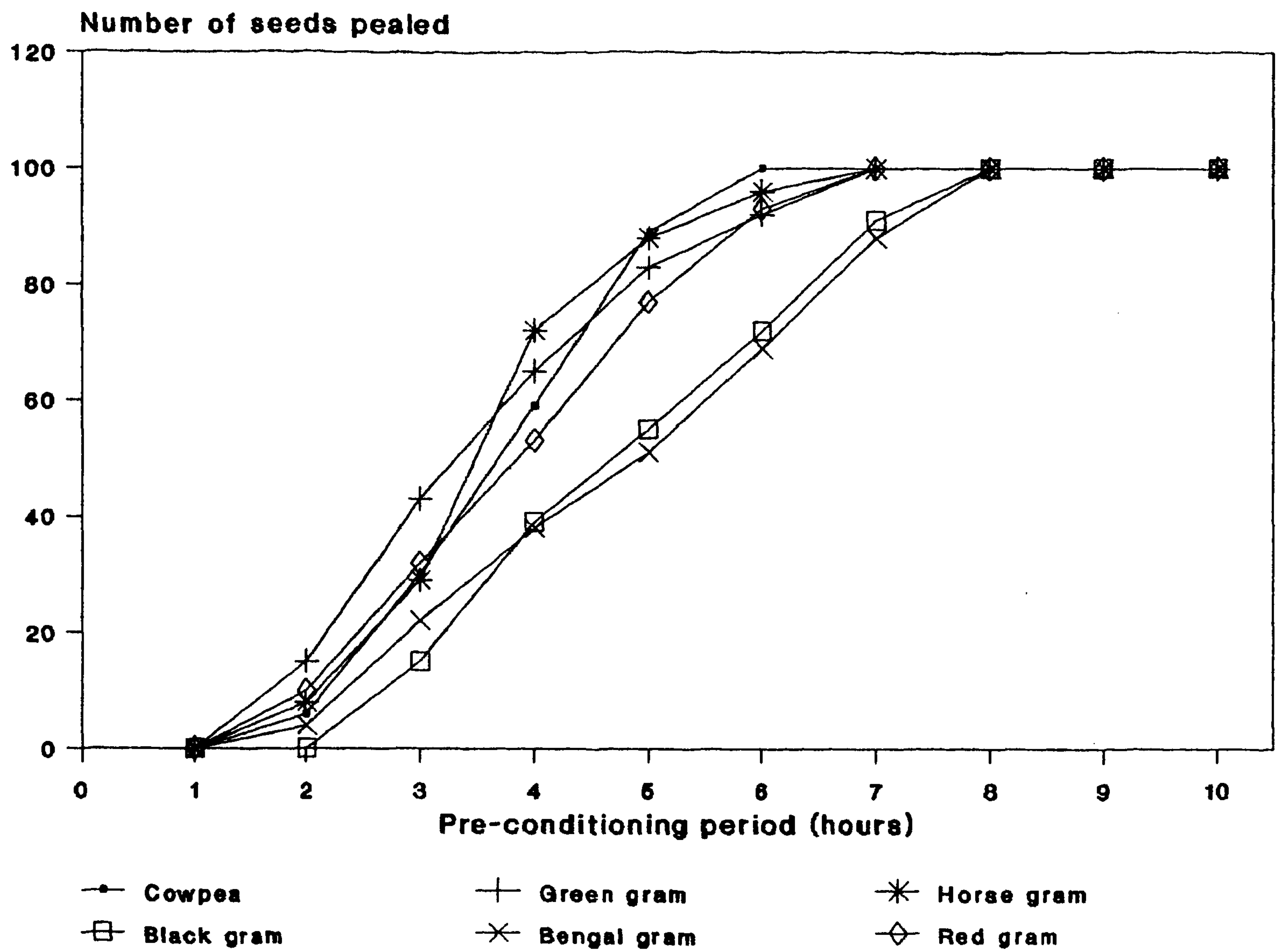

Fig. 1 Standardization of pre-conditioning period in six different pulse crop seeds

In cowpea the TZ reaction time varied from 2 hours to 22 hours depending on the TZ salt dilution and incubation temperature. At 25 ^{o}C incubation temperature, the reaction time was 22 hours for 0.10 per cent TZ solution concentration and it was reduced to three hours 30 minutes in 1.0 per cent concentration at the same temperature of incubation. At 30 ^{o}C and 0.10 per cent dilution of TZ solution the reaction time was 17 hours and it was reduced to three hours in one per cent TZ solution. At 35 ^{o}C incubation temperature and 0.10 per cent dilution the reaction time was 14 hours whereas at 1.0 per cent dilution the time required was 2 hours 30 minutes. In case of 40 ^{o}C incubation temperature, the reaction time was 12 hours at 0.10 per cent dilution and 2 hours at 1.0 per cent concentration of the solution (Table 2 and Fig.2a).

For greengram, the TZ reaction time varied from 2 hours to 24 hours depending on the TZ salt dilution and the incubation temperature. At 25 ^{o}C, the reaction time was 24 hours at 0.10 per cent TZ solution whereas it was 5 hours 30 minutes at 1.00 per cent TZ solution concentration. At 30 ^{o}C, it took 17 hours at 0.10 per cent TZ solution dilution and it took 2 hours 30 minutes at 1.00 per cent TZ dilution. At 35 ^{o}C, the reaction time was 13 hours at 0.10 per cent and 2 hours 15 minutes at 1.00 per cent TZ solution dilution. At 40 ^{o}C the reaction time was 5 hours at 0.10 per cent dilution and just two hours at 1.00 per cent TZ solution dilution (Table 2 and Fig.2a).

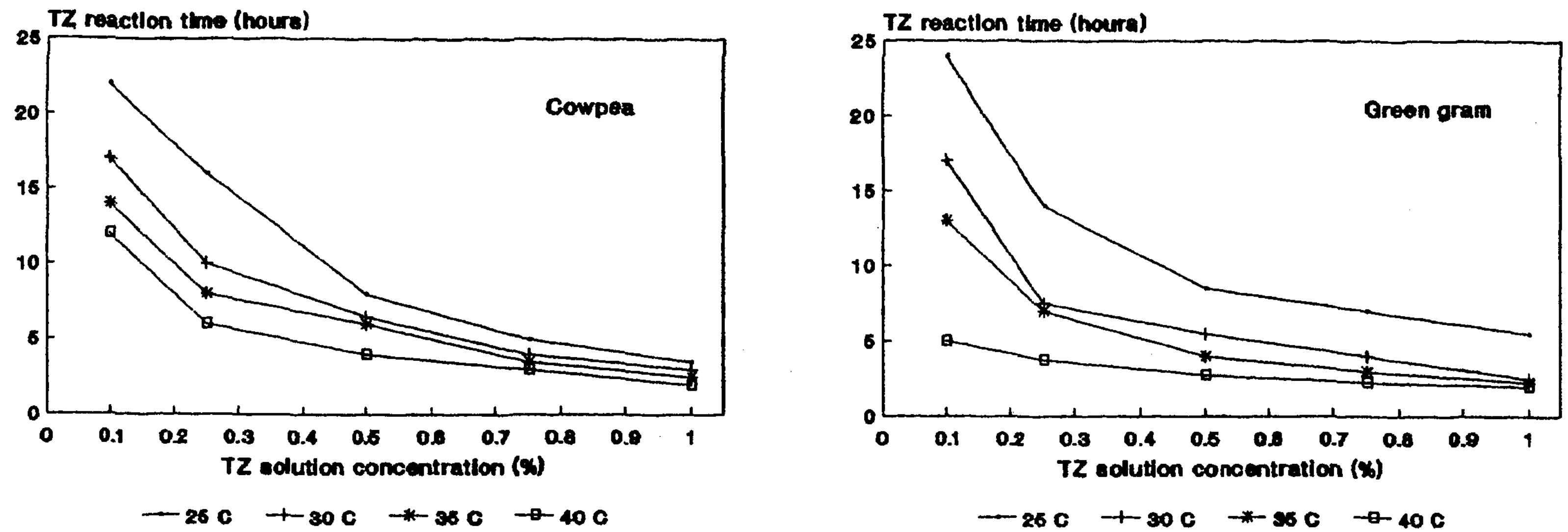

Fig 2a TZ reaction time for cowpea and green gram seeds at different incubation temperatures and different TZ solution concentrations

In horsegram, the TZ reaction time varied from 2 hours to 18 hours depending on the incubation temperature and TZ dilutions. At 25 °C and 0.10 per cent TZ solution dilution the reaction time was 18 hours whereas at 1.00 per cent TZ solution dilution it was 4 hours 30 minutes. At 30 °C, the reaction time was 16 hours at 0.10 per cent dilution and it was 3 hours 30 minutes at 1.00 per cent dilution. At 35 °C, the reaction time was 13 hours at 0.10 per cent dilution whereas at 1.00 per cent dilution it was 2 hours 45 minutes. At 40 °C, the reaction time at 0.10 per cent dilution was 11 hours and at 1.00 per cent TZ solution dilution it was just 2 hours (Table 2 and Fig. 2b).

In blackgram, the TZ reaction time varied from 2 hours 15 minutes to 30 hours depending upon the incubation temperature and TZ solution concentration. It was found that at 25 °C, the reaction time was 30 hours at 0.10 per cent concentration and it reduced to 9 hours at 1.00 per cent concentration. At 30 °C, the reaction time at 0.10 per cent dilution was 16 hours, which reduced to 5 hours 15 minutes at 1.00 per cent dilution. At 35 °C, the reaction time was 14 hours at 0.10 per cent dilution whereas it was reduced to 3 hours 30 minutes at 1.00 per cent dilution. At 40 °C, and 0.10 per cent dilution the reaction time was 10 hours whereas at 1.00 per cent TZ salt concentration it was reduced to just 2 hours 15 minutes (Table 2 and Fig. 2b).

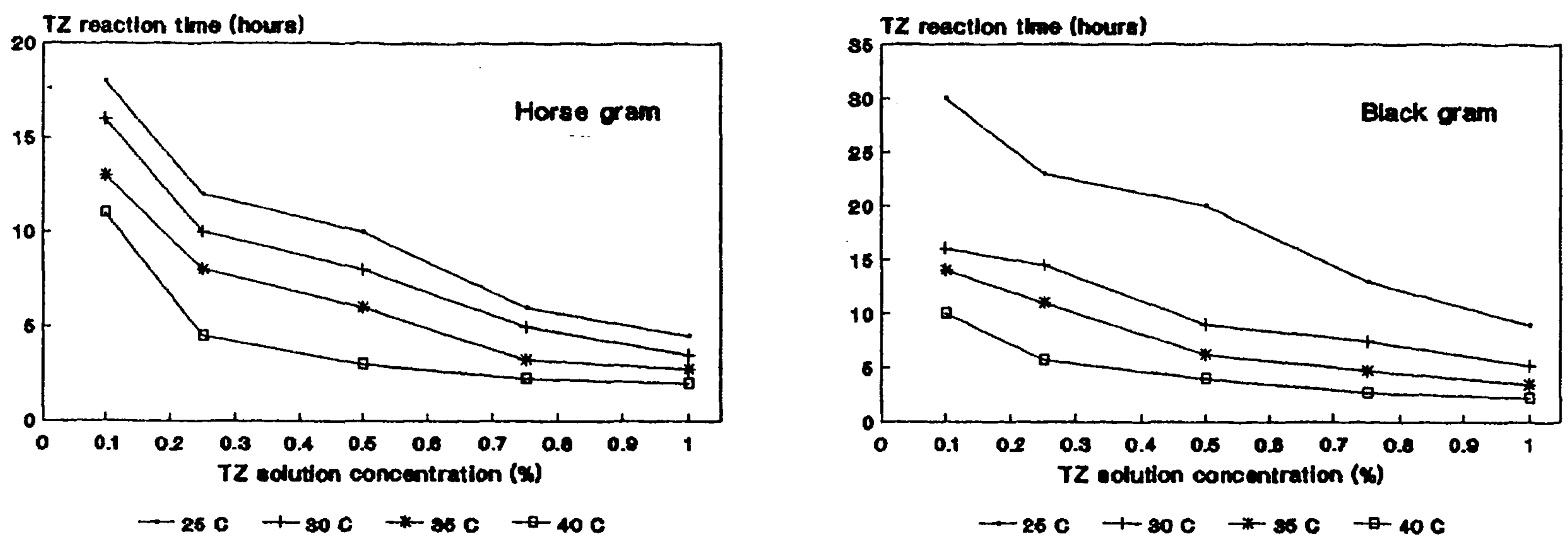

Fig 2b TZ reaction time for horse gram and black gram seeds at different incubation temperatures and different TZ solution concentrations

Table 2: Time taken for optimum staining of seeds as influenced by different incubation temperatures and TZ solution concentrations

Crop	Temperature (C)	Concentration (%)				
		0.10	0.25	0.50	0.75	1.00
		(---------hours and minutes------------)				
Cowpea	25	22.00	16.00	8.00	5.00	3.30
	30	17.00	10.00	6.30	4.00	3.00
	35	14.00	8.00	6.00	3.30	2.30
	40	12.00	6.00	4.00	3.00	2.00
Greengram	25	24.00	14.00	8.30	7.00	5.30
	30	17.00	7.30	5.30	4.00	2.30
	35	13.00	7.00	4.00	3.00	2.15
	40	5.00	3.45	2.45	2.15	2.00
Horsegram	25	18.00	12.00	10.00	6.00	4.30
	30	16.00	10.00	8.00	5.00	3.30
	35	13.00	8.00	6.00	3.15	2.45
	40	11.00	4.30	3.00	2.15	2.00
Blackgram	25	30.00	23.00	20.00	13.00	9.00
	30	16.00	14.30	9.00	7.30	5.15
	35	14.00	11.00	6.15	4.45	3.30
	40	10.00	5.45	4.00	2.45	2.15
Bengalgram	25	36.00	29.00	25.00	13.00	11.00
	30	21.00	15.00	12.00	9.00	6.45
	35	13.00	10.00	8.15	6.00	4.15
	40	6.15	4.30	3.15	2.45	2.00
Redgram	25	17.00	13.00	11.00	8.00	6.00
	30	14.00	11.00	9.00	7.00	5.00
	35	10.00	8.30	7.00	5.30	4.00
	40	9.00	7.00	6.00	4.30	3.30

In bengalgram, the TZ reaction time varied from 2 hours to 36 hours depending on the TZ salt dilution and incubation temperature. At 25 $^{\circ}$C incubation temperature, the reaction time was 36 hours at 0.10 per cent dilution whereas it was 11 hours at 1.0 per cent dilution. At 30 $^{\circ}$C incubation temperature, the reaction time was 21 hours at 0.10 per cent salt concentration but it was reduced to 6 hours 45 minutes at 1.00 per cent salt concentration. At 35 $^{\circ}$C, the reaction time was 13 hours at 0.10 per cent dilution and it was 4 hours 15 minutes at 1.00 per cent dilution. At 40 $^{\circ}$C, the reaction time was 6 hours 15 minutes at 0.10 per cent and 2 hours at 1.00 per cent TZ solution concentration (Table 2 and Fig. 2c).

In redgram, the TZ reaction time varied from 3 hours 30 minutes to 17 hours depending on TZ salt dilution and incubation temperature. At 25 $^{\circ}$C incubation temperature, the reaction time was 17 hours at 0.10 per cent concentration, whereas at 1.0 per cent dilution, it was 6 hours. At 30 $^{\circ}$C, the reaction time at 0.10 per cent dilution was 14 hours and at 1.0 per cent dilution it was 5 hours,. At 35 $^{\circ}$C, the reaction time was 10 hours at 0.10 per cent concentration, whereas at 1.0 per cent concentration it was 4 hours. At 40 $^{\circ}$C, the reaction time was 9 hours at 0.10 per cent dilution but it was reduced to 3 hours 30 minutes at 1.0 per cent dilution (Table 2 and Fig. 2c).

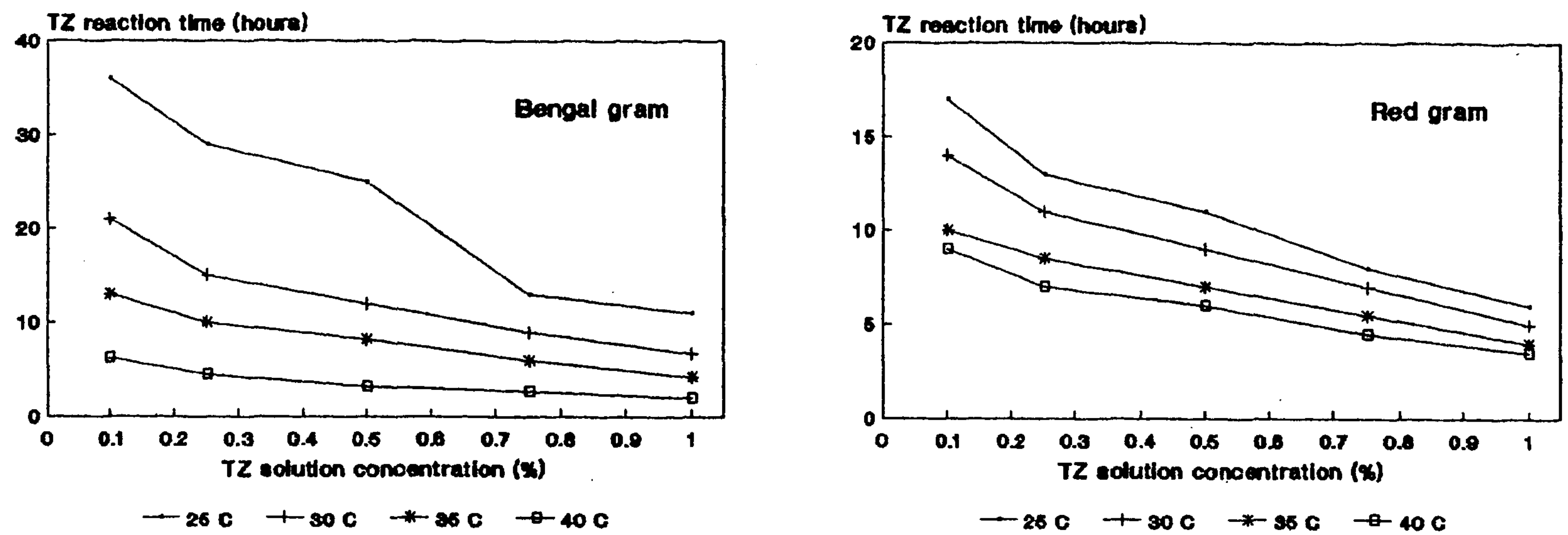

Fig 2c TZ reaction time for bengal gram and red gram seeds at different incubation temperatures and different TZ solution concentrations

4.3 Standard germination and field emergence test results

The results of standard germination and field emergence studies conducted for the six major pulse crops are presented in Table 3.

The viable seed percentage varied from crop to crop; which varied from 95.25 per cent (bengalgram) to 73.25 per cent (horsegram). The percentage field emergnece varied from 98 per cent (bengalgram) to 73.50 per cent (horsegram). Incowpea, the germination percentage was 92.50 and field emergence percentage was 89.00. In greengram the standard germination and field emergence were 93.25 and 88.50 per cent respectively. In horsegram the germination percentage was 73.25 and field emergence percentage was 73.50. In blackgram the germination was 89.25 per cent whereas field emergence was 84.50 per cent. In case of bengalgram, the standard germination and field emergence were 95.25 per cent and 98.00 per cent respectively. In redgram the viable seed percentage obtained in standard germination test was 91.25 and the field emergence was 77.00 per cent (Table 3).

4.4 TZ test results

The cropwise results of the comparative viability studies using different TZ solution concentrations and incubation temperatures are presented below.

4.4.1 Cowpea

In cowpea, the TZ staining patterns were grouped into 10 categories as germinable or non-germinable and the details are presented below.

Table 3: Comparative standard germination and field emergence test results

Crop	Variety	Standard germination (%)	Field emergence (%)
Cowpea	C-152	92.50	89.00
Greengram	Local	93.25	88.50
Horsegram	Local	73.25	73.50
Blackgram	T-9	89.25	84.50
Bengalgram	Local	95.25	98.00
Redgram	Hyd.3C	91.25	77.00

Categories

1. Embryo completely stained.
2. Embryo stained except for small unstained patches on the cotyledon opposite to the radicle.
3. Embryo stained except for small irregular unstained dots on the cotyledons.
4. Embryo stained except for unstained small patches on the periphery of the cotyledon.
5. Embryo stained except for small specks, nearly 10 per cent of the total area of a cotyledon opposite to the radicle being unstained.
6. Embryo stained but the radicle tip unstained.
7. Embryo stained except for small specks, nearly 10 per cent of the total area of a cotyledon near the radicle being unstained.
8. Embryo stained only in margin or surface and nearly 50 per cent unstained.
9. Embryo stained but the whole radicle unstained.
10. Embryo insect attacked.

Based on the earlier research findings, categories one to seven were classified as 'Germinable' and the rest were 'Non-germinable'. The percentage values of seeds falling under each categories are presented in Table 4.

In cowpea at 25 ^{o}C incubation temperature, in all the TZ solutions more than 50 per cent of the seeds falling in the first category and up to 15.5 per cent in case of second category. Similarly in 30 ^{o}C, the first category recorded more than 50 per cent of seeds and in the second category it was up to 14.50 per cent. Again in 35 ^{o}C, more than 50 per cent of the seeds falling in first category and in case of

TZ staining patterns in cowpea

1. Embryo completely stained.

2. Embryo stained except for small irregular unstained dots on the cotyledons.

3. Embryo stained but the radicle tip unstained.

4. Embryo stained except for small unstained patches on the cotyledon opposite to the radicle.

5. Embryo stained except for small specks, nearly 10 per cent of the total area of a cotyledon opposite to the radicle being unstained.

6. Embryo stained except for small specks, nearly 25 per cent of the total area of a cotyledon opposite to the radicle being unstained.

7. Embryo stained except for small specks, nearly 10 per cent of the total area of a cotyledon near the radicle being unstained.

8. Embryo stained but the whole radicle unstained.

9. Embryo stained only in margin or surface and nearly 50 per cent unstained.

10. Embryo completely unstained

11. Embryo insect attacked.

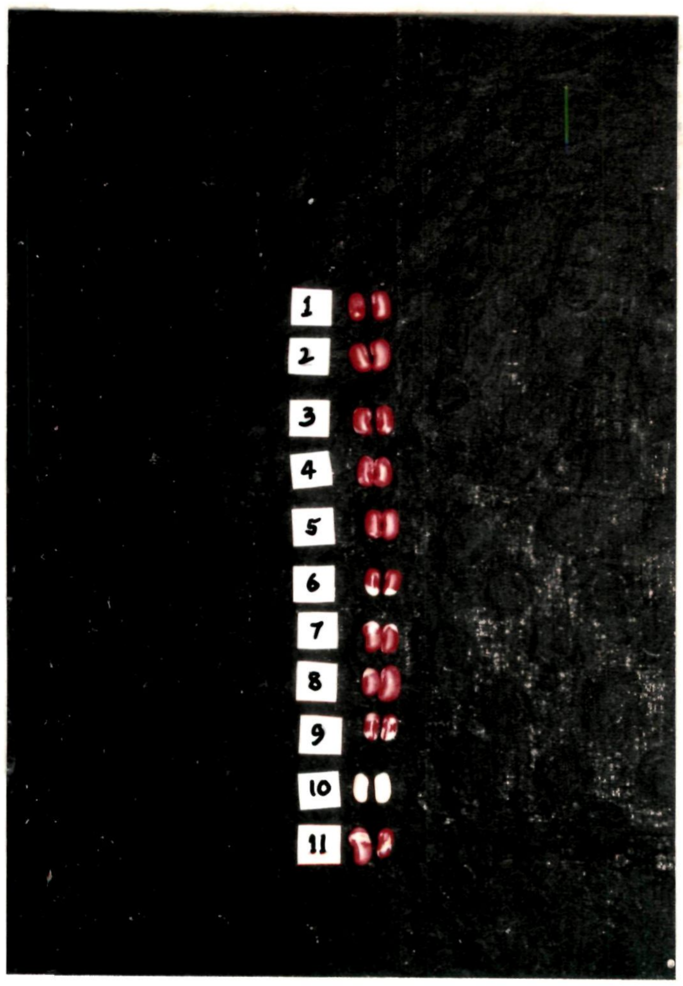

Plate 1: TZ staining patterns in cowpea (1,2,3,4,5,6 and 7 germinable; 8,9,10 and 11 non-germinable)

second it was up to 17 per cent. In 40 ^{o}C, the first category has more than 50 per cent of total seeds while the second has up to 15 per cent.

Among the 't' values for the different category combinations, the summarised least 't' values for different TZ dilution and incubation temperatures are also presented in Table 4 which indicate the most feasible category combinations representing the viable seed categories. The comparative viability studies in cowpea using TZ test, standard germination test and field emergence are presented in Table 5. Here the treatments of 25 ^{o}C incubation temperature in 0.1 per cent TZ solution dilution and 0.75 per cent as well as 1.00 per cent TZ solution dilutions at 40 ^{o}C incubation temperature treatments found to differ significantly from other treatments as according to 't' test (Table 4).

In 25 ^{o}C, the per cent seed viability varied from 87.5 to 93.5 per cent. In 0.10 per cent dilution the per cent viable seed was 87.5 whereas it was 93.5 per cent in 0.25 per cent dilution. In 30 ^{o}C, the viable seed percentage varied from 87.5 per cent in case of 0.50 per cent dilution to 91 per cent in case of 0.10 per cent TZ solution dilution. In case of 35 ^{o}C, the lower and upper values of viable seed percentage was 88.5 per cent in 1.0 per cent dilution and 92 per cent in 0.25 per cent dilution, respectively. In 40 ^{o}C,

Table 4: Seeds of cowpea falling under different categories after TZ treatment and the combinations given least 't' values

Temperature (°C)	Concentration (%)	Categories 1	2	3	4	5	6	7	8	9	10
	0.10	73.0	7.0	3.5	0.5	2.0	1.0	0.5	8.0	1.5	3.0
	0.25	63.0	15.5	0.0	3.5	6.0	2.5	3.0	6.0	0.0	0.5
25	0.50	62.0	14.5	2.0	2.0	6.0	1.0	2.0	7.5	1.5	1.5
	0.75	58.0	14.0	8.5	1.0	5.0	2.5	0.5	9.5	1.0	0.0
	1.00	60.5	14.5	5.0	3.0	5.5	1.0	1.5	8.0	0.0	1.0
	0.10	70.0	12.0	3.0	0.5	2.0	0.0	3.5	8.0	0.5	0.5
	0.25	62.0	14.5	2.0	2.5	4.0	1.5	1.5	8.5	2.0	1.5
30	0.50	60.0	13.5	4.5	2.5	5.0	0.0	2.0	8.5	0.5	3.5
	0.75	61.5	13.0	8.5	0.0	4.5	1.0	2.0	8.5	0.0	1.0
	1.00	60.5	14.0	5.5	0.5	7.0	1.0	2.0	8.5	0.0	1.0
	0.10	67.5	15.0	0.5	0.5	4.0	1.5	0.0	8.0	1.5	1.5
	0.25	62.0	17.0	1.5	3.0	6.0	0.5	2.0	6.0	0.5	1.5
35	0.50	62.5	13.5	1.5	2.5	7.0	1.5	2.0	8.0	0.5	1.0
	0.75	68.5	10.0	4.5	1.5	3.5	0.0	1.5	8.0	1.0	1.5
	1.00	61.5	14.0	3.5	2.5	4.5	1.0	1.5	8.5	0.5	2.5
	0.10	64.0	15.0	2.5	0.0	5.0	1.0	1.5	7.0	0.5	3.5
	0.25	67.0	14.0	2.5	3.0	1.5	1.5	1.0	7.5	0.5	1.5
40	0.50	64.0	10.5	6.0	1.0	5.0	0.5	1.0	1.0	0.5	0.5
	0.75	59.0	12.0	9.5	1.0	5.0	0.0	1.0	10.5	0.0	2.0
	1.00	54.5	12.0	8.5	1.5	7.5	1.0	1.0	10.5	1.0	2.5

('t' table continued)

Standard germination (%) 92.50
Field emergence (%) 89.00

Category combinations given least 't' values in cowpea

Temperature (°C)	TZ concentration (%)	Categories	't' value
25	0.10	1,2,3,4,5,6 and 7	3.118*
	0.25	1,2,3,4,5,6 and 7	0.225
	0.50	1,2,3,4,5,6 and 7	1.225
	0.75	1,2,3,4,5,6 and 7	0.923
	1.00	1,2,3,4,5,6 and 7	0.281
30	0.10	1,2,3,4,5,6 and 7	0.635
	0.25	1,2,3,4,5,6 and 7	1.375
	0.50	1,2,3,4,5,6 and 7	1.871
	0.75	1,2,3,4,5,6 and 7	1.038
	1.00	1,2,3,4,5,6 and 7	1.038
35	0.10	1,2,3,4,5,6 and 7	0.685
	0.25	1,2,3,4,5,6 and 7	0.153
	0.50	1,2,3,4,5,6 and 7	0.346
	0.75	1,2,3,4,5,6 and 7	0.676
	1.00	1,2,3,4,5,6 and 7	0.876
40	0.10	1,2,3,4,5,6 and 7	0.754
	0.25	1,2,3,4,5,6 and 7	1.038
	0.50	1,2,3,4,5,6 and 7	1.291
	0.75	1,2,3,4,5,6 and 7	2.269*
	1.00	1,2,3,4,5,6 and 7	2.239*

Table 't' value (P = 0.05) = 2.145

Table 5: Comparative seed viability in cowpea using TZ test, standard germination and field emergence tests

TZ solution Concentration (%)	Incubation temperature (°C)			
	25 °C	30 °C	35 °C	40 °C
	(---------------Viable seed (%)---------------)			
0.10	87.5*	91.0	89.0	89.0
0.25	93.5	88.0	92.0	90.5
0.50	89.5	87.5	90.5	88.0
0.75	89.5	90.5	89.5	87.5*
1.00	91.0	90.5	88.5	86.0*

Seed viability by standard germination test: 92.50 %
Field emergence: 89.00 %

* Significant at $P = 0.05$; as according to 't' test

incubation temperature, the per cent viable seeds varied from 86 per cent in case of 1.0 per cent dilution to 90.5 per cent in 0.25 per cent TZ salt solution.

4.4.2 Greengram

In Greengram, the topographical staining patterns were grouped into 17 categories after TZ treatment, which are presented as below.

Categories

1. Embryo stained cherry red
2. Embryo stained with intermediary colour to cherry red and pink.
3. Embryo stained pink.
4. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon, opposite to the radicle being unstained.
5. Embryo stained but having small scattered dark spots.
6. Embryo stained except for small, scattered unstained dots on the cotyledons.
7. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon, near the radicle being unstained.
8. Embryo stained except for an unstained dot on the radicle.
9. Embryo stained except for thin unstained streaks on the cotyledon.
10. Embryo stained but the radicle tip unstained.
11. Embryo stained but the basal part of radicle unstained.
12. Embryo stained except for a patch, nearly 25 per cent of total area of a cotyledon opposite to the radicle being unstained.
13. Embryo stained except for a patch, nearly 25 per cent of the total area of a cotyledon near the radicle being unstained.

TZ staining patterns in greengram

1. Embryo stained with intermediary colour to cherry red and pink.

2. Embryo stained pink.

3. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon, near the radicle being unstained.

4. Embryo stained but the radicle tip unstained.

5. Embryo stained but the basal part of radicle unstained.

6. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon, opposite to the radicle being unstained.

7. Embryo stained but the whole radicle unstained.

8. Embryo stained but having small scattered dark spots.

9. Embryo stained except for thin unstained streaks on the cotyledon.

10. Embryo stained except for a patch, nearly 25 per cent of total area of a cotyledon opposite to the radicle being unstained.

11. Embryo stained except for a patch, nearly 25 per cent of the total area of a cotyledon near the radicle being unstained.

12. Embryo insect attacked.

13. Embryo stained only in surface or periphery and nearly 50 per cent unstained

14. Embryo completely unstained.

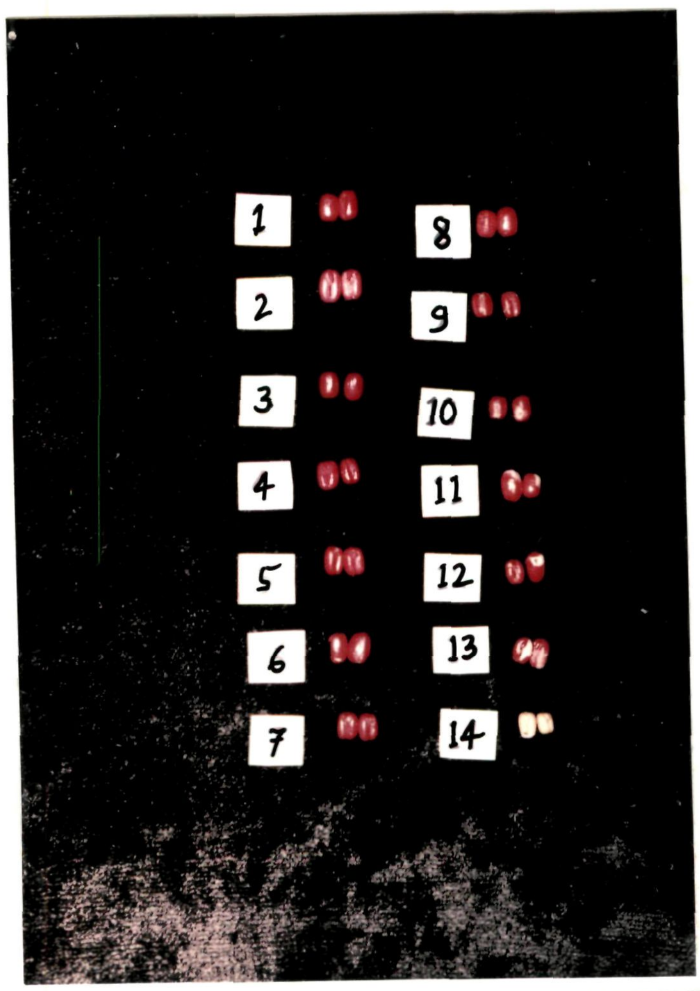

Plate 2: TZ staining patterns in greengram (1,2,3,4,5,6,8 9,10 andll germinable;7,12,13 and 14 non-germinable)

14. Embryo stained only in margin or surface and nearly 50 per cent unstained.

15. Embryo stained but the whole radicle unstained.

16. Embryo completely unstained.

17. Embryo insect attacked.

Based on the earlier research findings the categories 1 to 13 were classified as 'Germinable' and the rest were 'Non-germinable'. The percentage values of seeds falling under each categories are presented in Table 6.

In 25°C, the third category with 0.10 per cent TZ solution concentration recorded more than 50 per cent of seeds whereas the second category had 22 per cent of total seeds. At 25°C, in all other higher TZ concentration treatments, it was the second category containing more than 50 per cent of the total seeds. In 30°C, at 0.10 per cent ofTZ dilution again the third category had more than 50 per cent of seeds whereas 24 per cent of the seeds were falling under the second category. In all other higher TZ concentrations the second category had more than 50 per cent of the total seeds. In 35°C, the categories 2 and 3 jointly contributed to more than 50 per cent of seeds in case of 0.10, 0.25 and 0.50 per cent TZ dilutions whereas at 0.75 and 1.00 per cent dilutions, the second category alone had more than 50 per cent of seeds to fall under that category. In 40°C, the second category contained more than 50 per cent of the seeds except in 1.00 per cent dilution, which had 44.50 per cent seeds under that category (Table 6).

Table 6: Seeds of greengram falling under different categories after TZ treatment and the combinations given least 't' values

Temperature (°C)	Concentration (%)	Categories 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	0.10	0.0	22.0	62.0	1.0	4.0	1.0	0.5	0.0	0.0	0.0	3.5	0.0	0.0	4.0	0.0	0.5	1.5
	0.25	0.5	66.6	8.5	1.5	4.5	3.0	3.0	2.0	0.5	2.0	2.5	0.5	1.0	1.5	2.0	0.0	0.5
25	0.50	1.0	65.0	4.0	4.0	9.0	0.5	2.0	0.5	1.0	1.5	3.0	0.5	1.5	3.5	0.5	0.0	2.5
	0.75	0.0	67.0	2.0	3.5	3.5	2.5	2.0	1.5	1.0	2.5	3.0	0.5	2.5	4.0	3.0	0.0	1.5
	1.00	0.0	66.0	5.0	2.0	7.0	1.5	4.5	4.5	0.5	0.5	2.5	0.0	0.0	2.0	1.0	0.5	2.5
	0.10	1.0	24.0	59.0	1.5	4.5	1.5	0.0	0.0	0.0	0.0	2.5	0.0	0.0	4.5	0.0	0.0	1.5
	0.25	0.0	60.5	0.5	1.0	4.5	4.5	1.5	6.5	2.0	2.0	6.0	0.5	2.0	3.0	1.5	2.0	2.0
30	0.50	2.0	69.5	0.0	1.0	4.0	3.5	2.5	2.0	0.5	2.0	4.5	0.5	0.0	2.0	2.5	1.0	2.5
	0.75	1.5	64.5	4.0	2.0	5.0	3.5	4.0	3.5	1.0	2.0	4.0	0.0	0.5	1.0	2.5	0.0	1.0
	1.00	0.0	64.0	5.0	3.0	3.0	4.0	4.0	4.0	2.0	3.5	2.0	0.5	0.0	2.0	1.5	1.5	0.0
	0.10	2.0	48.0	15.0	0.0	11.5	3.0	3.0	4.5	4.0	0.5	3.0	0.5	0.5	2.5	1.0	0.5	0.5
	0.25	0.0	42.0	8.5	3.0	20.0	4.0	2.0	2.5	4.0	1.5	1.5	0.5	1.0	7.0	1.0	1.5	0.0
35	0.50	0.0	45.0	6.5	1.5	20.5	2.0	5.5	0.5	4.0	1.5	3.0	1.0	1.0	4.0	3.0	0.0	1.0
	0.75	0.0	56.5	8.5	3.5	10.0	2.5	2.0	3.0	1.5	1.5	3.0	1.0	0.5	5.0	1.5	0.0	0.0
	1.00	0.0	61.5	6.5	2.5	9.0	2.0	3.5	3.0	2.5	1.0	1.5	0.5	1.0	3.0	1.5	1.0	0.0
	0.10	4.0	53.0	6.5	0.5	7.0	2.5	4.5	4.0	2.0	1.0	5.5	0.0	0.0	3.5	2.0	2.0	2.0
	0.25	9.5	50.0	4.5	1.5	12.0	2.0	3.5	1.5	0.5	2.0	4.0	0.0	1.0	4.0	2.0	1.0	1.0
40	0.50	5.5	55.5	0.5	4.5	5.0	3.5	4.0	5.0	0.5	1.0	1.5	0.5	0.5	4.0	3.5	3.0	2.0
	0.75	7.5	52.5	2.5	2.0	5.0	2.5	0.5	5.0	2.0	1.5	4.5	0.5	2.0	4.0	6.5	0.5	1.0
	1.00	10.5	44.5	1.0	2.5	8.5	3.5	3.5	2.5	3.0	0.5	7.0	1.0	2.0	5.0	4.5	0.0	0.5

('t' table continued)

Standard germination (%) 93.25
Field emergence (%) 88.50

Category combinations given least 't' values in greengram

Temperature (°C)	TZ concentration (%)	Categories	't' value
25	0.10	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.191
	0.25	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.701
	0.50	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.053
	0.75	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.307
	1.00	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.168
30	0.10	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.168
	0.25	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.363
	0.50	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.232
	0.75	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.599
	1.00	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.455
35	0.10	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.521
	0.25	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.704
	0.50	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.252
	0.75	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.060
	1.00	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.281
40	0.10	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.532
	0.25	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.265
	0.50	1,2,3,4,5,6,7,8,9,10,11,12 and 13	1.193
	0.75	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.875
	1.00	1,2,3,4,5,6,7,8,9,10,11,12 and 13	0.580

Table 't' value (P = 0.05) = 2.145

Table 7: Comparative seed viability in greengram using TZ test, standard germination and field emergence tests

TZ solution Concentration (%)	Incubation temperature (^{o}C)			
	$25^{o}C$	$30^{o}C$	$35^{o}C$	$40^{o}C$
	(Viable seed (%))			
0.10	94.0	94.0	95.5	90.5
0.25	96.0	91.5	90.5	92.0
0.50	93.5	92.0	92.0	87.5
0.75	91.5	95.5	93.5	88.0
1.00	94.0	95.0	94.5	90.0

Seed viability by standard germination test: 93.25 %
Field emergence: 88.50 %

4. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon opposite to the radicle being unstained.

5. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon near the radicle being unstained.

6. Embryo stained but having small scattered dark spots.

7. Embryo stained except for thin unstained streaks on the cotyledons.

8. Embryo stained except for small unstained dots scattered on the cotyledons.

9. Embryo stained but the basal part of the radicle unstained.

10. Embryo stained but the radicle tip unstained.

11. Embryo stained except for a patch, nearly 25 per cent of the total area of the cotyledon opposite to the radicle being unstained.

12. Embryo stained except for a patch, nearly 25 per cent of the total area of the cotyledon near the radicle being unstained.

13. Embryo insect attacked.

14. Embryo stained but the whole radicle unstained.

15. Embryo stained only in margin or surface and nearly 50 per cent unstained.

16. Embryo completely unstained.

Based on earlier research findings, the categories 1 to 11 were classified as 'Germinable' and the rest were 'Non-germinable'. The percentage values of seeds falling under each categories are presented in Table 8.

In 25^{o}C, the second and third categories jointly contributed for more than 50 per cent of the total seeds

TZ staining patterns in horsegram

1. Embryo stained cherry red.
2. Embryo stained pink.
3. Embryo stained with intermediary colour to cherry red and pink.
4. Embryo stained but the basal part of the radicle unstained.
5. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon near the radicle being unstained.
6. Embryo insect attacked.
7. Embryo stained except for thin unstained streaks on the cotyledons.
8. Embryo stained but the whole radicle unstained.
9. Embryo stained except for a patch, nearly 25 per cent of the total area of the cotyledon opposite to the radicle being unstained.
10. Embryo stained except for a patch, nearly 25 per cent of the total area of the cotyledon near the radicle being unstained.
11. Embryo stained only in margin or surface and nearly 50 per cent unstained.
12. Embryo completely unstained.

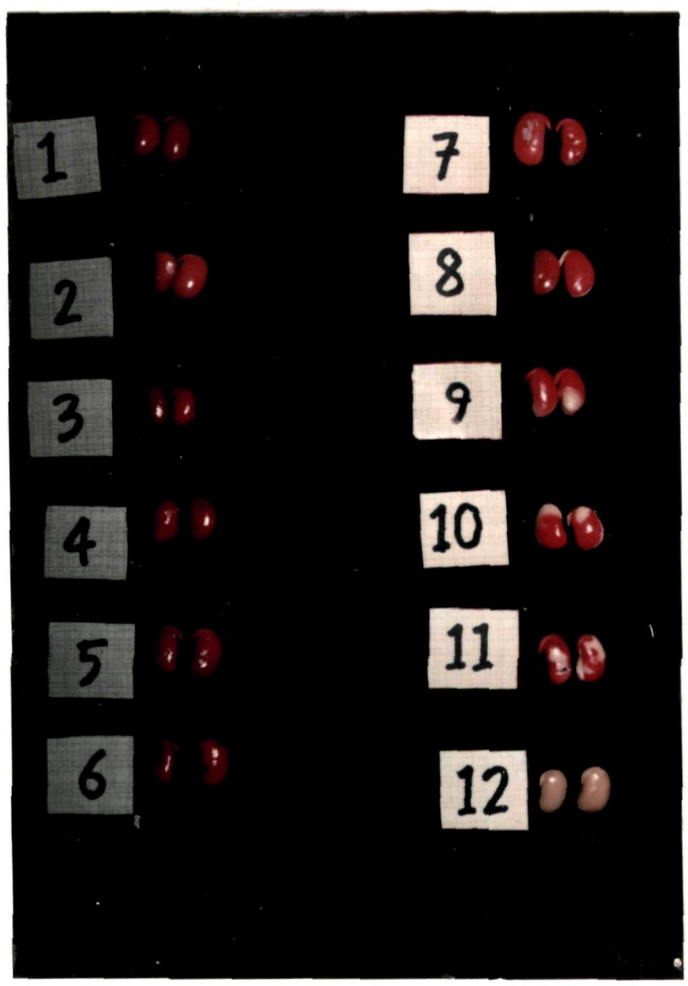

Plate 3: TZ staining patterns in horsegram (1,2,3,4,5,7 and 9 germinable; 6,8,10,11 and 12 non-germinable)

except in case of 0.50 per cent dilution, which has 48.50 per cent seeds. The sixth category contained 11 to 16.5 per cent of the seeds in different concentration levels. In $30^{\circ}C$, again the second and third categories jointly contained more than 50 per cent of seeds except in case of 0.75 and 1.00 per cent TZ concentrations, which had the values 44.5 per cent and 49.50 per cent respectively. The sixth category contained 10 to 16 per cent of the total seeds among the different TZ dilution levels. In $35^{\circ}C$ incubation temperature, the second category contained 31 to 44 per cent of seeds and the third category has the seeds in the range of 0 to 15.5 per cent among the different levels of TZ dilutions. The sixth category contained 9.5 to 14.5 per cent of the total seeds and the second category contained 33 to 43.5 per cent of seeds in different TZ concentration levels.

The least 't' values were obtained for the category combinations of 1 to 11, which are also pres ted in Table 8. The comparative viability studies in horsegram using TZ test, standard germination and field emergence are presented in Table 9.

In $25^{\circ}C$, the per cent seed viability varied from 79.5 per cent in 0.10 per cent dilution to 84.5 per cent in 0.75 per cent dilution. In $30^{\circ}C$, it varied from 75 per cent incase of 0.75 per cent TZ dilution to 87 per cent in 0.25 per cent dilution. In $35^{\circ}C$, it ranged from 74 to 80 per cent in case of 0.50 per cent, 0.10 per cent and 0.25 per cent

Table 8:Seeds of horsegram falling under different categories after TZ treatment and the combinations given least 't' values

Temperature (°C)	Concentration (%)	Categories 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	0.10	4.5	19.0	32.0	0.5	0.0	15.0	4.5	2.5	1.0	0.0	0.5	1.0	2.0	5.5	6.0	6.0
	0.25	1.0	35.5	20.0	0.5	0.5	14.5	6.0	1.0	1.5	0.0	0.5	1.5	4.0	3.5	4.5	5.5
25	0.50	3.5	40.0	8.5	1.0	0.5	11.0	9.0	4.5	1.0	0.5	0.5	0.5	3.5	4.5	5.5	6.0
	0.75	0.5	40.0	11.5	2.5	1.0	19.0	5.5	4.0	0.0	0.0	0.5	0.0	1.0	3.0	4.0	7.5
	1.00	0.0	47.0	10.5	1.5	0.5	16.5	2.5	1.5	1.5	0.0	1.0	1.0	3.0	4.0	3.0	6.5
	0.10	3.0	43.0	20.0	3.5	1.0	10.0	2.0	1.0	0.5	0.0	0.0	0.5	3.0	3.0	5.0	4.5
	0.25	8.0	39.5	16.0	0.0	0.0	16.0	3.0	3.5	0.5	0.5	0.0	1.0	3.0	3.5	3.0	2.5
30	0.50	5.5	44.5	6.5	2.0	4.0	13.0	3.5	2.0	0.0	0.5	0.5	1.0	4.0	3.0	3.0	7.0
	0.75	7.0	38.0	6.5	3.0	0.0	12.0	4.0	3.0	1.0	0.0	0.5	1.0	3.5	7.5	6.5	6.5
	1.00	4.0	45.5	4.0	0.5	1.0	14.5	3.0	3.5	0.5	1.0	0.0	1.0	1.0	8.0	6.5	6.0
	0.10	5.0	44.0	10.5	2.5	1.5	9.5	2.0	2.5	0.5	1.0	1.0	0.5	1.5	4.5	9.0	4.5
	0.25	5.5	31.0	15.5	3.0	1.0	13.5	3.0	5.0	1.5	0.0	1.0	2.0	5.5	5.0	6.0	1.5
35	0.50	1.0	40.5	6.5	1.5	1.0	13.5	4.0	4.0	1.0	0.0	1.0	1.0	5.0	3.5	6.5	10.0
	0.75	10.0	36.0	0.0	1.5	2.5	14.5	3.5	7.0	1.5	1.0	0.0	0.0	3.0	3.0	6.5	10.0
	1.00	6.5	38.0	0.5	2.0	2.0	14.5	4.0	5.5	2.5	0.5	1.5	1.0	4.5	3.5	7.5	6.0
	0.10	12.5	33.0	9.0	1.5	0.5	6.5	5.0	4.0	2.0	0.0	1.5	0.5	4.5	6.5	5.0	8.0
	0.25	9.5	41.5	4.5	2.0	1.0	8.5	3.0	3.5	1.0	0.0	0.5	1.0	2.5	5.0	9.0	7.5
40	0.50	14.0	39.0	0.5	2.5	0.0	10.5	2.0	5.5	0.0	0.0	2.0	1.5	4.5	7.5	4.0	6.0
	0.75	11.5	43.5	0.5	1.0	2.5	7.5	2.0	2.5	2.0	0.0	2.5	1.0	4.5	4.0	7.0	8.0
	1.00	10.5	38.0	0.0	2.0	1.5	9.5	6.0	7.5	1.0	0.0	1.5	1.0	4.0	4.5	7.0	6.0

('t' table continued)

Standard germination (%) 73.25
Field emergence (%) 73.50

Category combinations given least 't' values in horsegram

Temperature (°C)	TZ concentration (%)	Categories	't' value
	0.10	1,2,3,4,5,6,7,8,9,10 and 11	0.858
	0.25	1,2,3,4,5,6,7,8,9,10 and 11	1.940
25	0.50	1,2,3,4,5,6,7,8,9,10 and 11	1.076
	0.75	1,2,3,4,5,6,7,8,9,10 and 11	2.013
	1.00	1,2,3,4,5,6,7,8,9,10 and 11	1.362
	0.10	1,2,3,4,5,6,7,8,9,10 and 11	1.252
	0.25	1,2,3,4,5,6,7,8,9,10 and 11	1.744
30	0.50	1,2,3,4,5,6,7,8,9,10 and 11	1.394
	0.75	1,2,3,4,5,6,7,8,9,10 and 11	0.321
	1.00	1,2,3,4,5,6,7,8,9,10 and 11	0.626
	0.10	1,2,3,4,5,6,7,8,9,10 and 11	0.947
	0.25	1,2,3,4,5,6,7,8,9,10 and 11	1.018
35	0.50	1,2,3,4,5,6,7,8,9,10 and 11	0.110
	0.75	1,2,3,4,5,6,7,8,9,10 and 11	0.699
	1.00	1,2,3,4,5,6,7,8,9,10 and 11	0.512
	0.10	1,2,3,4,5,6,7,8,9,10 and 11	0.320
	0.25	1,2,3,4,5,6,7,8,9,10 and 11	0.473
40	0.50	1,2,3,4,5,6,7,8,9,10 and 11	0.520
	0.75	1,2,3,4,5,6,7,8,9,10 and 11	0.403
	1.00	1,2,3,4,5,6,7,8,9,10 and 11	0.660

Table 't' value (P = 0.05) = 2.145

Table 9 :Comparative seed viability in horsegram using TZ test, standard germination and field emergence tests

TZ solution Concentration (%)	Incubation temperature (°C)			
	25 °C	30 °C	35 °C	40 °C
	(Viable seed (%))			
0.10	79.5	84.0	80.0	75.5
0.25	81.0	87.0	80.0	75.0
0.50	80.0	82.0	74.0	76.0
0.75	84.5	75.0	77.5	75.5
1.00	82.5	77.5	77.5	77.5

Seed viability by standard germination test: 73.25 %
Field emergence: 73.50 %

respectively. In $40^{o}C$ incubation temperature the viable seed percentage varied from 75 per cent in 0.25 per cent TZ dilution to 77.5 per cent in 1.00 per cent TZ dilution.

4.4.4 Blackgram

In Blackgram, the topographical staining patterns were grouped into 13 categories which are presented as under.

Categories

1. Embryo stained cherry red.
2. Embryo stained with intermediary colour to cherry red and pink.
3. Embryo stained pink.
4. Embryo stained but having small scattered dark spots.
5. Embryo stained but having thin dark streaks here and there.
6. Embryo stained except for small specks, nearly 10 per cent of the total area of cotyledon oppostite to the radicle being unstained.
7. Embryo stained except for small specks, nearly 10 per cent of the total area of cotyledon near the radicle part being unstained.
8. Embryo stained except for a patch, nearly 25 per cent of the total area of cotyledon opposite to the radicle being unstained.
9. Embryo stained except for a patch nearly 25 per cent of the total area of cotyledon near the radicle being unstained.
10. Embryo stained only in margin or surface and nearly 50 per cent untained.
11. Embryo stained but the radicle unstained.
12. Embryo completely unstained.
13. Embryo insect attacked.

TZ staining patterns in blackgram

1. Embryo stained with intermediary colour to cherry red and pink.

2. Embryo stained pink.

3. Embryo stained cherry red.

4. Embryo stained except for small specks, nearly 10 per cent of the total area of cotyledon near the radicle part being unstained.

5. Embryo stained except for small specks, nearly 10 per cent of the total area of cotyledon oppostite to the radicle being unstained.

6. Embryo stained except for a patch, nearly 25 per cent of the total area of cotyledon opposite to the radicle being unstained.

7. Embryo stained but having small scattered dark spots.

8. Embryo stained but having thin dark streaks here and there.

9. Embryo stained but the radicle unstained.

10. Embryo stained only in margin or surface and nearly 50 per cent untained.

11. Embryo insect attacked.

12. Embryo completely unstained.

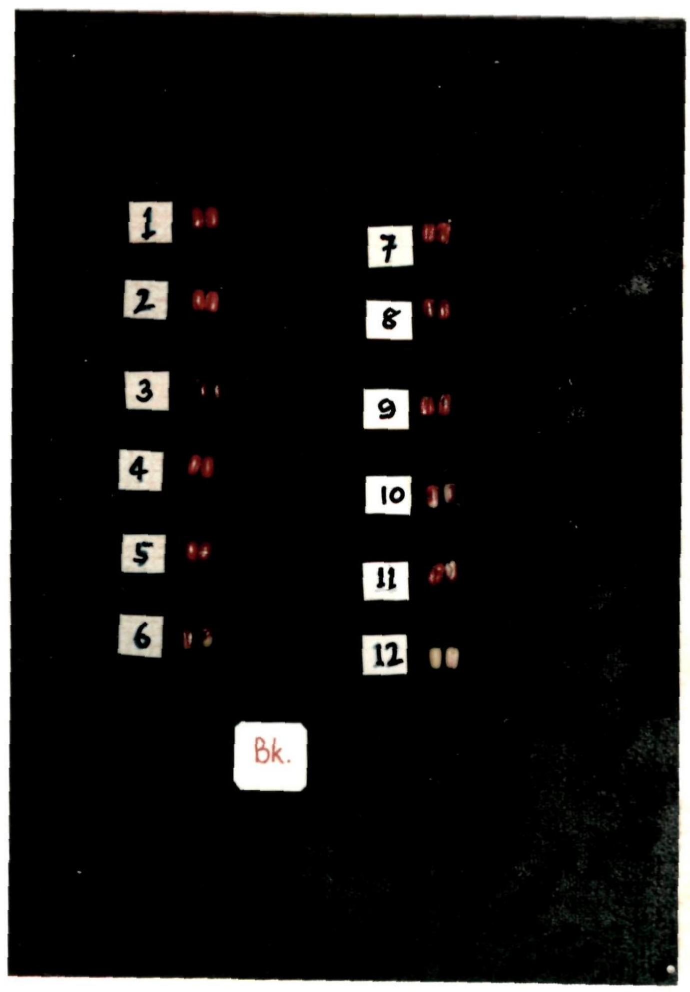

Plate 4: TZ staining patterns in blackgram (1,2,3,4,5,6,7 and 8 germinable; 9,10,11 and 12 non-germinable)

Based on earlier research findings, the categories 1 to 9 were found to be 'Germinable' and the rest were 'Non-germinable'. The percentage values of seeds falling iunder each categories are presented in Table 10.

In 25 ^{o}C, the seeds falling under category 2 had the values from 18 to 42 per cent and in the third category the values ranged from 9 to 48.5 per cent whereas in the 4th category the values were in the range of 13 to 23.5 per cent. In 30 ^{o}C, the values in the second category are in the range of 11 to 44.5 per cent, and in third category they are in the range of 11 to 58 per cent and in fourth category the per cent seed values varied from 13.5 to 23 per cent. In case of 35 ^{o}C incubation temperature, the values in the first category are ranging from 1 to 22 per cent and in the second it is 11 to 46 per cent and in the third category it has the range of 6.5 to 60.5 per cent whereas in fourth category the values varied from 10.5 to 21.5 per cent. In 40 ^{o}C, the number of seeds falling in second category ranged from 29.5 to 52.5 per cent and in the third category the values ranged from 9.5 to 36.5 per cent and in the 4th category it ranged from 13 to 26.5 per cent.

The least 't' values were obtained for the category combinations of 1 to 9, which are also presented in Table 10. The comparative viability studies in blackgram using TZ test, standard germination and field emergence tests are presented in Table 11.

Table 10: Seeds of blackgram falling under different categories after TZ treatment and the combinations given least 't' values

Temperature (°C)	Concentration (%)	Categories 1	2	3	4	5	6	7	8	9	10	11	12	13
	0.10	0.0	18.0	48.5	19.0	6.0	0.0	0.0	0.0	1.0	1.5	0.5	4.0	1.5
	0.25	1.0	28.5	40.0	13.0	4.0	1.0	0.0	1.0	1.5	1.5	0.0	7.5	1.0
25	0.50	6.0	37.0	15.5	23.5	6.5	0.5	0.0	0.0	0.5	3.5	0.0	6.5	0.5
	0.75	11.0	42.0	9.0	17.5	7.0	1.5	1.0	0.0	0.0	4.0	1.5	4.5	1.0
	1.00	4.5	35.0	23.5	21.0	8.5	0.0	0.0	0 0	0 0	1.5	1.5	5.0	0.0
	0.10	1.0	11.0	58.0	17.0	4.5	0.0	0.0	0.0	0.0	3.0	0.0	5.0	0.5
	0.25	8.5	42.0	16.5	13.5	6.5	1.0	0.0	0.5	0.0	4.0	2.0	5.0	0.5
30	0.50	13.0	36.0	14.0	17.0	8.0	0.0	0.0	0.0	0.0	1.5	1.5	9.0	0.0
	0.75	3.5	43.5	11.0	23.0	7.0	0.5	0.0	1.5	0.0	3.5	0.5	6.0	0.0
	1.00	3.0	44.5	11.0	18.5	4.0	1.0	0.5	0.5	0.0	6.0	1.5	8.0	1.5
	0.10	1.0	11.0	60.5	12.5	6.0	0.0	0.0	0.0	0.0	3.0	0.0	5.0	1.0
	0.25	4.0	42.0	13.0	21.5	6.0	1.0	0.0	2.0	0.0	4.0	0.5	6.0	0.0
35	0.50	22.0	34.5	10.5	10.5	16.5	0.0	0.0	0.0	0.0	0.5	0.5	4.5	0.5
	0.75	19.0	36.5	12.0	13.5	10.0	0.5	0.0	0.0	1.0	3.0	0.5	3.5	0.5
	1.00	18.5	46.0	6.5	12.5	5.5	0.0	0.0	0.0	0.0	4.0	2.5	4.0	0.5
	0.10	6.5	29.5	36.5	8.5	4.5	0.0	0.0	0.0	2.0	2.5	0.0	8.5	1.5
	0.25	7.5	33.5	13.5	18.5	13.0	1.0	0.0	0.0	1.5	3.5	1.0	7.0	0.0
40	0.50	10.0	32.0	10.0	26.5	11.0	0.0	0.0	0.5	0.0	2.5	1.5	5.5	0.5
	0.75	13.0	35.0	13.5	17.5	7.5	1.0	1.0	0.0	0.5	5.0	0.5	4.5	1.0
	1.00	11.5	52.5	9.5	13.0	0.0	0.5	0.0	0.0	1.5	3.5	0.5	7.5	0.0

('t' table continued)

Standard germination (%) 89.25
Field emergence (%) 84.55

Category combinations given least 't' values in blackgram

Temperature (°C)	TZ concentration (%)	Categories	't' value
	0.10	1,2,3,4,5,6,7,8 and 9	0.789
	0.25	1,2,3,4,5,6,7,8 and 9	0.188
25	0.50	1,2,3,4,5,6,7,8 and 9	0.053
	0.75	1,2,3,4,5,6,7,8 and 9	0.053
	1.00	1,2,3,4,5,6,7,8 and 9	0.741
	0.10	1,2,3,4,5,6,7,8 and 9	0.440
	0.25	1,2,3,4,5,6,7,8 and 9	0.119
30	0.50	1,2,3,4,5,6,7,8 and 9	0.214
	0.75	1,2,3,4,5,6,7,8 and 9	0.150
	1.00	1,2,3,4,5,6,7,8 and 9	1.116
	0.10	1,2,3,4,5,6,7,8 and 9	0.338
	0.25	1,2,3,4,5,6,7,8 and 9	0.053
35	0.50	1,2,3,4,5,6,7,8 and 9	1.112
	0.75	1,2,3,4,5,6,7,8 and 9	0.700
	1.00	1,2,3,4,5,6,7,8 and 9	0.051
	0.10	1,2,3,4,5,6,7,8 and 9	0.305
	0.25	1,2,3,4,5,6,7,8 and 9	0.154
40	0.50	1,2,3,4,5,6,7,8 and 9	0.176
	0.75	1,2,3,4,5,6,7,8 and 9	0.056
	1.00	1,2,3,4,5,6,7,8 and 9	0.162

Table 't' value (P = 0.05) = 2.145

Table 11: Comparative seed viability in blackgram using TZ test, standard germination and field emergence tests

TZ solution Concentration (%)	Incubation temperature (^{o}C)			
	25 ^{o}C	30 ^{o}C	35 ^{o}C	40 ^{o}C
	(Viable seed (%))			
0.10	92.5	91.5	91.0	87.5
0.25	90.0	88.5	89.5	88.5
0.50	89.5	88.0	94.0	90.0
0.75	89.0	90.0	92.5	89.0
1.00	92.5	83.5	89.0	88.5

Seed viability by standard germination test: 89.25 %
Field emergence: 84.50 %

In 25 ^{o}C the viable seed values varied from 89 per cent in case of 0.75 per cent to 92.5 per cent in case of 0.10 per cent and 1.00 per cent TZ dilutions. In 30 ^{o}C, the values ranged from 83.5 per cent in 1.0 per cent dilution to 91.5 per cent in 0.10 per cent dilution. In 35 ^{o}C, the viable seed per cent varied from 89 per cent in 1.0 per cent dilution to 94 per cent in 0.5 per cent dilution. In 40 ^{o}C, the values ranged from 87.5 per cent in 0.10 per cent dilution to 90 per cent in 0.50 per cent TZ solution.

4.4.5 Bengalgram

In bengalgram, the topographical staining patterns were grouped into 13 categories which are presented as under:

Categories

1. Embryo stained cherry red.
2. Embryo stained with intermediary colour to cherry red and pink.
3. Embryo stained pink.
4. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon near the radicle unstained.
5. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledons opposite to the radicle unstained.
6. Embryo stained except for small unstained scattered dots on the cotyledons.
7. Embryo stained but the radicle tip unstained.
8. Embryo stained except for thin unstained streaks on the cotyledons.
9. Embryo stained but having scattered small dark spots.

TZ staining patterns in bengalgram

1. Embryo stained with intermediary colour to cherry red and pink.
2. Embryo stained pink.
3. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledons opposite to the radicle unstained.
4. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon near the radicle unstained.
5. Embryo stained except for small unstained scattered dots on the cotyledons.
6. Embryo stained except for thin unstained streaks on the cotyledons.
7. Embryo stained but the radicle tip unstained.
8. Embryo stained only in margin / surface and nearly 50 % unstained.
9. Embryo insect attacked
10. Embryo stained cherry red.

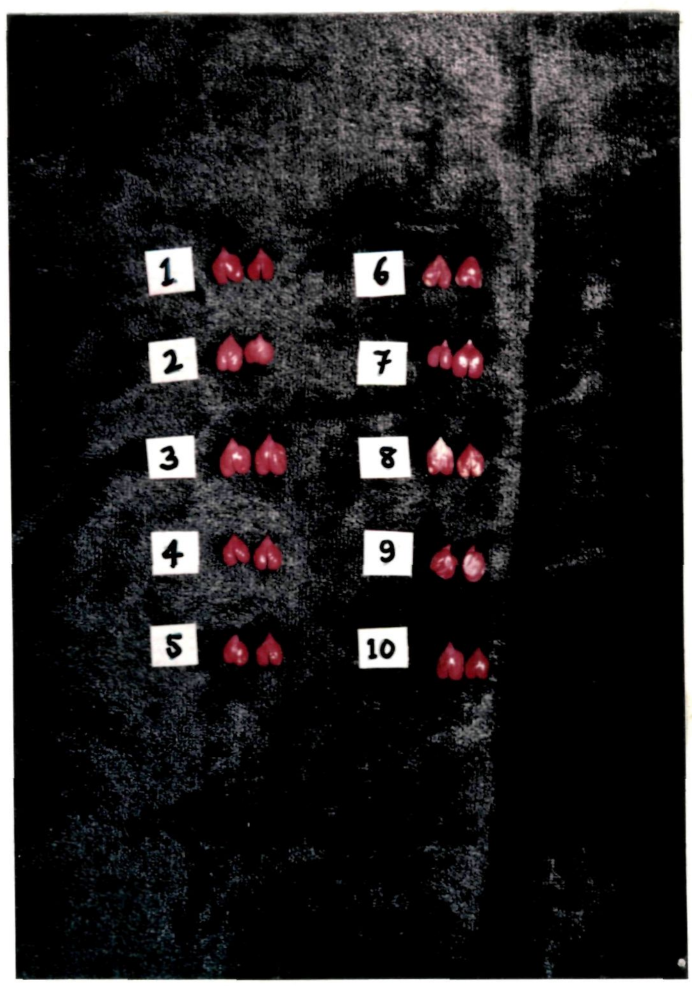

Plate 5: TZ staining patterns in bengalgram (1,2,3,4,5,6,7 and 10 germinable; 8 and 9 non-germinable)

10. Embryo stained but having dark patches here and there.

11. Embryo stained only in margin or surface and nearly 50 per cent unstained.

12. Embryo completely unstained.

13. Embryo insect attacked.

From the earlier research findings, categories 1 to 10 were classified as 'Germinable' and the rest were 'non-germinable'. The percentage values of seeds falling under each categories are presented in Table 12.

In 25 °C incubation temperature the second and third categories jointly contained more than 50 per cent of the seeds, similarly in 30 °C, all but 1.0 per cent TZ solution dilution was having more than 50 per cent of seeds. In 35 °C, again the second and third categories jointly contained more than 50 per cent of the seeds; whereas in 40 °C, the second category alone contained more than 50 per cent of the seeds in all the TZ dilution treatments.

The least 't' values in case of bengalgram were obtained for the category combinations which are presented in Table 12 itself. The comparative viability studies in bengalgram using TZ test, standard germination and field emergence tests are presented in Table 13.

In 25 °C, the viable seeds varied from 94.5 to 98.5 per cent in the TZ dilutions of 1.00 per cent and 0.10 per cent respectively. In 30 °C, the values ranged from 93 per cent as in case of 0.5 and 0.75 per cent dilutions to 97 per cent in

Table 12: Seeds of bengalgram falling under different **categories after** TZ **treatment** and the combinations given least 't' values

Temperature (°C)	Concentration (%)	Categories 1	2	3	4	5	6	7	8	9	10	11	12	13
	0.10	1.0	27.5	51.5	2.5	5.0	7.5	0.0	1.0	2.5	0.0	0.5	0.5	0.5
	0.25	1.0	65.5	8.5	2.0	7.0	6.0	1.0	3.0	2.5	0.0	1.5	0.5	1.5
25	0.50	3.5	50.5	13.5	2.5	7.5	7.5	1.0	2.5	5.5	2.5	2.0	0.5	1.0
	0.75	3.5	57.5	8.0	2.0	4.5	8.5	0.0	4.5	7.5	1.5	1.5	1.0	0.0
	1.00	5.5	53.0	6.0	2.0	5.0	6.0	1.0	4.0	6.5	5.5	2.5	1.5	1.5
	0.10	0.0	22.5	55.5	0.5	5.5	9.0	0.0	1.0	0.0	0.0	1.5	2.5	2.0
	0.25	1.5	50.0	23.0	2.0	5.0	8.0	0.0	3.0	4.0	0.5	1.5	0.5	1.0
30	0.50	2.0	60.5	2.0	3.0	4.0	9.5	1.0	3.0	6.5	1.5	1.5	1.0	4.5
	0.75	2.0	57.0	3.5	1.5	5.5	10.5	1.0	4.5	7.5	0.5	2.5	0.5	4.5
	1.00	3.5	43.5	4.5	5.0	8.0	10.0	1.5	5.0	11.0	4.0	2.0	1.0	1.0
	0.10	0.5	21.5	54.5	2.0	6.0	6.0	1.0	2.0	1.5	0.0	1.0	2.0	2.0
	0.25	2.5	56.0	10.0	3.0	8.0	6.5	2.5	3.0	1.0	0.0	4.0	0.5	3.0
35	0.50	4.0	53.0	8.5	7.0	7.5	4.5	2.5	4.0	4.0	0.0	4.0	0.5	0.5
	0.75	1.5	55.5	10.0	3.5	6.0	6.0	1.0	4.5	5.5	0.0	4.0	0.5	2.0
	1.00	1.0	44.0	9.0	6.0	4.5	14.0	2.5	3.5	10.0	0.5	4.5	0.0	0.5
	0.10	0.0	64.0	0.0	3.5	14.0	9.5	2.5	2.5	0.0	0.0	2.0	0.5	1.5
	0.25	0.0	63.5	0.0	3.0	13.0	8.0	1.0	5.0	0.0	0.0	4.0	0.5	2.0
40	0.50	0.0	62.0	0.0	7.0	11.0	7.5	2.0	6.0	0.0	0.0	2.5	1.5	0.5
	0.75	1.5	57.5	5.0	5.0	8.5	6.0	1.5	4.5	3.5	0.0	4.5	1.5	1.0
	1.00	2.0	51.0	5.5	4.0	9.5	13.0	3.5	4.0	3.0	0.5	2.5	1.0	1.5

('t' table continued)

Standard germination (%) 95.25
Field emergence (%) 98.00

Category combinations given least 't' values in bengalgram

Temperature (°C)	TZ concentration (%)	Categories	't' value
25	0.10	1,2,3,4,5,6,7,8,9 and 10	1.381
	0.25	1,2,3,4,5,6,7,8,9 and 10	0.418
	0.50	1,2,3,4,5,6,7,8,9 and 10	0.418
	0.75	1,2,3,4,5,6,7,8,9 and 10	0.805
	1.00	1,2,3,4,5,6,7,8,9 and 10	0.213
30	0.10	1,2,3,4,5,6,7,8,9 and 10	0.443
	0.25	1,2,3,4,5,6,7,8,9 and 10	0.644
	0.50	1,2,3,4,5,6,7,8,9 and 10	0.723
	0.75	1,2,3,4,5,6,7,8,9 and 10	0.596
	1.00	1,2,3,4,5,6,7,8,9 and 10	0.266
35	0.10	1,2,3,4,5,6,7,8,9 and 10	0.092
	0.25	1,2,3,4,5,6,7,8,9 and 10	0.748
	0.50	1,2,3,4,5,6,7,8,9 and 10	0.080
	0.75	1,2,3,4,5,6,7,8,9 and 10	0.744
	1.00	1,2,3,4,5,6,7,8,9 and 10	0.066
40	0.10	1,2,3,4,5,6,7,8,9 and 10	0.266
	0.25	1,2,3,4,5,6,7,8,9 and 10	0.496
	0.50	1,2,3,4,5,6,7,8,9 and 10	0.084
	0.75	1,2,3,4,5,6,7,8,9 and 10	0.650
	1.00	1,2,3,4,5,6,7,8,9 and 10	0.315

Table 't' value (P = 0.05) = 2.145

Table 13: Comparative seed viability in bengalgram using TZ test, standard germination and field emergence tests

TZ solution Concentration (%)	Incubation temperature (^{o}C)			
	$25^{o}C$	$30^{o}C$	$35^{o}C$	$40^{o}C$
	(Viable seed (%))			
0.10	98.5	94.0	95.0	96.0
0.25	96.5	97.0	92.5	93.5
0.50	96.5	93.0	95.0	95.5
0.75	97.5	93.0	93.5	93.0
1.00	94.5	96.0	95.0	96.0

Seed viability by standard germination test: 95.25 %
Field emergence: 98.00 %

case of 0.25 per cent dilution. In 35 °C, the viable seeds had the range of 92.5 per cent in case of 0.25 per cent dilution to 95 per cent in case of 0.10 per cent or 0.50 per cent or 1.00 per cent dilutions. In 40 °C incubation temperature, the viable seed per cent ranged from 93 per cent in case of 0.75 per cent dilution to 96 per cent in case of 0.10 per cent and 1.00 per cent respectively.

4.4.6 **Redgram**

In Redgram, the topographical staining patterns were grouped into 13 categories after TZ staining which are presented below.

Categories

1. Embryo stained cherry red.
2. Embryo stained with intermediary colour to cherry red and pink.
3. Embryo stained but having thin unstained streaks on the cotyledons.
4. Embryo stained but having scattered small unstained spots on the cotyledons.
5. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon on the radicle part being unstained.
6. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon opposite to the radicle being unstained.
7. Embryo stained but having small black patches scattered on the cotyledons.
8. Embryo stained but the radicle tip unstained.
9. Embryo stained except for a patch, nearly 25 per cent of the total area of the cotyledon near the radicle being unstained.

TZ staining patterns in redgram

1. Embryo stained cherry red.
2. Embryo stained with intermediary colour to cherry red and pink.
3. Embryo stained but having scattered small unstained spots
4. Embryo stained but having thin unstained streaks on the cotyledons.
5. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon opposite to the radicle being unstained.
6. Embryo stained except for small specks, nearly 10 per cent of the total area of the cotyledon on the radicle part being unstained.
7. Embryo stained but the radicle tip unstained.
8. Embryo stained except for a patch, nearly 25 per cent of the total area of the cotyledon near the radicle being unstained.
9. Embryo stained except for a patch, nearly 25 per cent of the cotyledon opposite to the radicle being unstained.
10. Embryo stained only in margin or surface and nearly 50 per cent unstained.
11. Embryo insect attacked.
12. Embryo completely unstained.

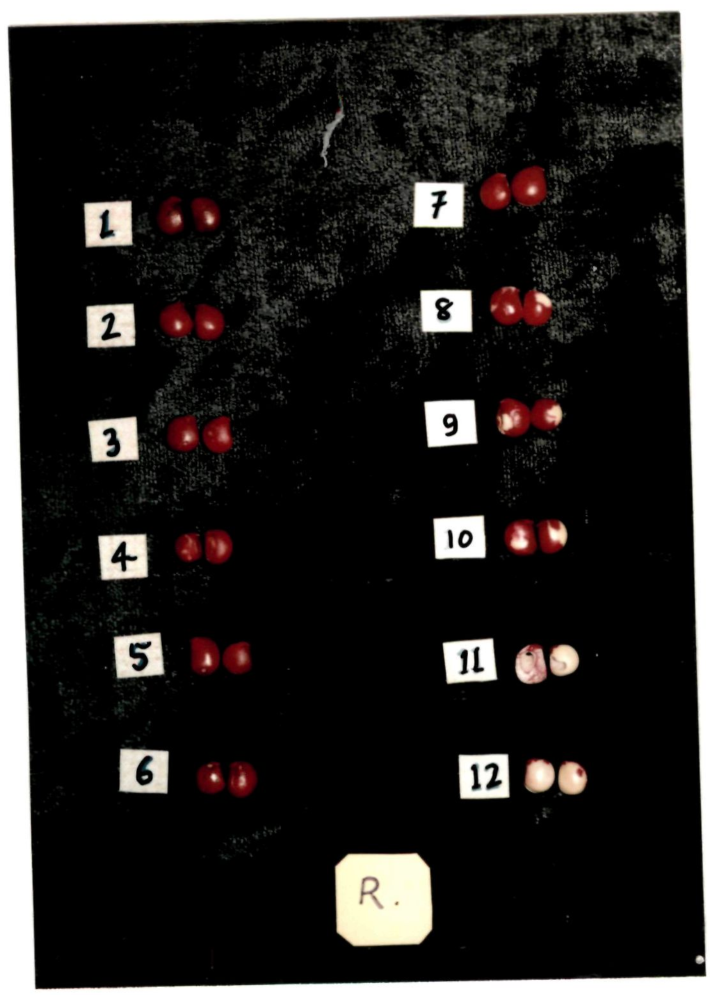

Plate 6: TZ staining patterns in redgram (1,2,3,4,5,6,7,8 and 9 germinable; 10, 11 and 12 non-germinable)

10. Embryo stained except for a patch, nearly 25 per cent of the cotyledon opposite to the radicle being unstained.

11. Embryo stained only in margin or surface and nearly 50 per cent unstained.

12. Embryo completely unstained.

13. Embryo insect attacked.

Based on earlier research findings, the categories 1 to 10 were found to be 'Germinable' an the rest were 'Non-germinable'. The percentage values of seeds falling under each categories are presented in Table 14.

In 25 ^{o}C incubation temperature, among the different TZ dilution treatments the percentage of seeds falling under second category ranged from 36.50 per cent to 63.50 per cent and in case of seventh category it ranged from 13.5 to 26.5 per cent and in the eleventh category the range was 8 to 17 per cent. In 30 ^{o}C, the percentage of seeds falling under second category varied from 38 to 52.5 per cent and in the seventh category it ranged from 13.5 to 30 per cent whereas in the 11th category the range was 9.5 to 11.5 per cent. In 35 ^{o}C, incubation temperature, the percentage of seeds falling under second category ranged from 44 to 59.5 per cent and in the seventh category it varied from 7 to 23.5 per cent. In case of 40 ^{o}C, the second category contained more than 50 per cent of seeds compared to other categories. The least 't' values in case of redgram were obtained for the category combinations of 1 to 10 which are also resented in table 14. The comparative viability studies in redgram using

Plate 7: Standard germination and TZ test in cowpea

1. Normal seedlings (93%)
2. Abnormal seedlings (4%)
3. Dead seeds (3%)
4. TZ treated seeds (germinable: 93%)
(non-germinable: 7%)

Plate 8: Field emergence test in cowpea (89%)

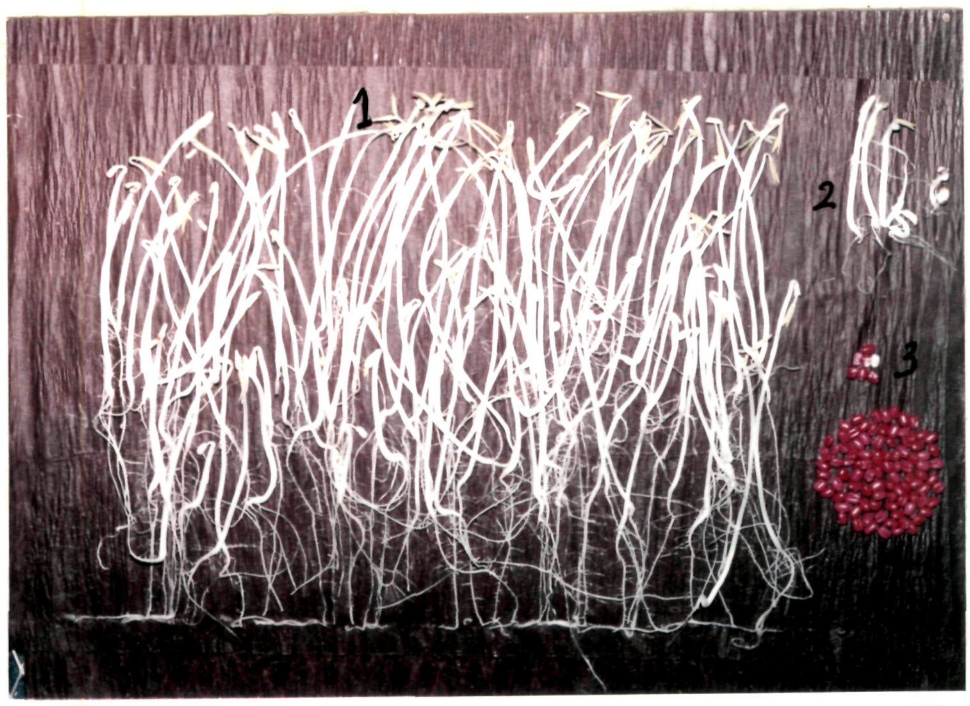

Plate 9: Standard germination and TZ test in greengram

1. Normal seedlings (94%)
2. Abnormal seedlings (6%)
3. TZ treated seeds (non germinable: 7%)
4. TZ treated seeds (germinable: 93%)

Plate 10:Field emergence test in greengram (85%)

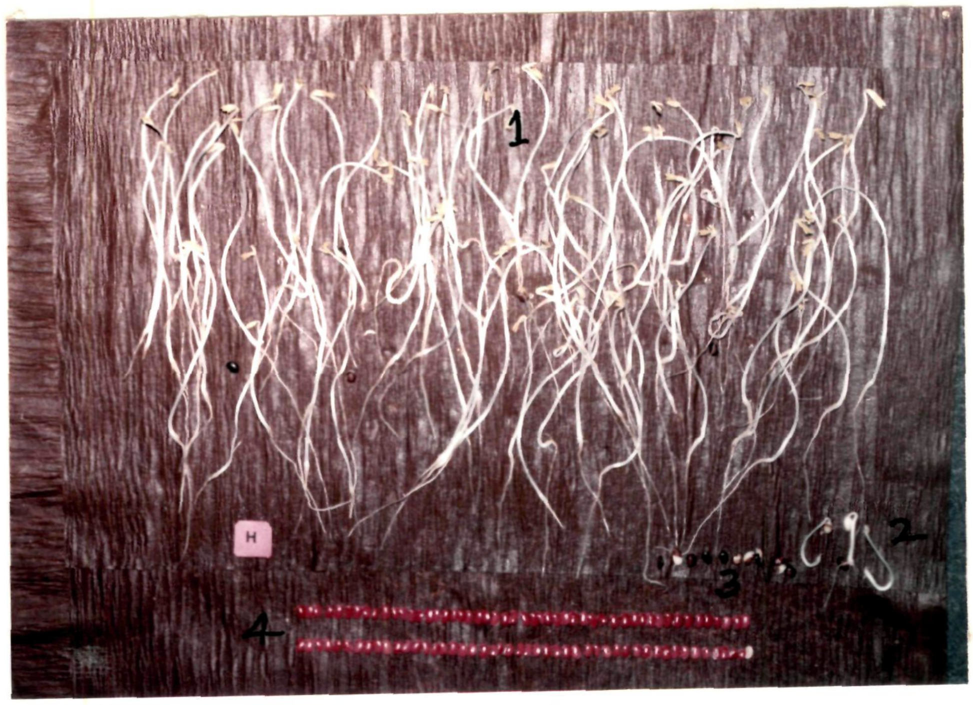

Plate 11: Standard germination and TZ test in horsegram

1. Normal seedlings (86%)
2. Abnormal seedlings (3%)
3. Dead seeds (11%)
4. TZ treated seeds (germinable: 88%)
(non-germinable: 12%)

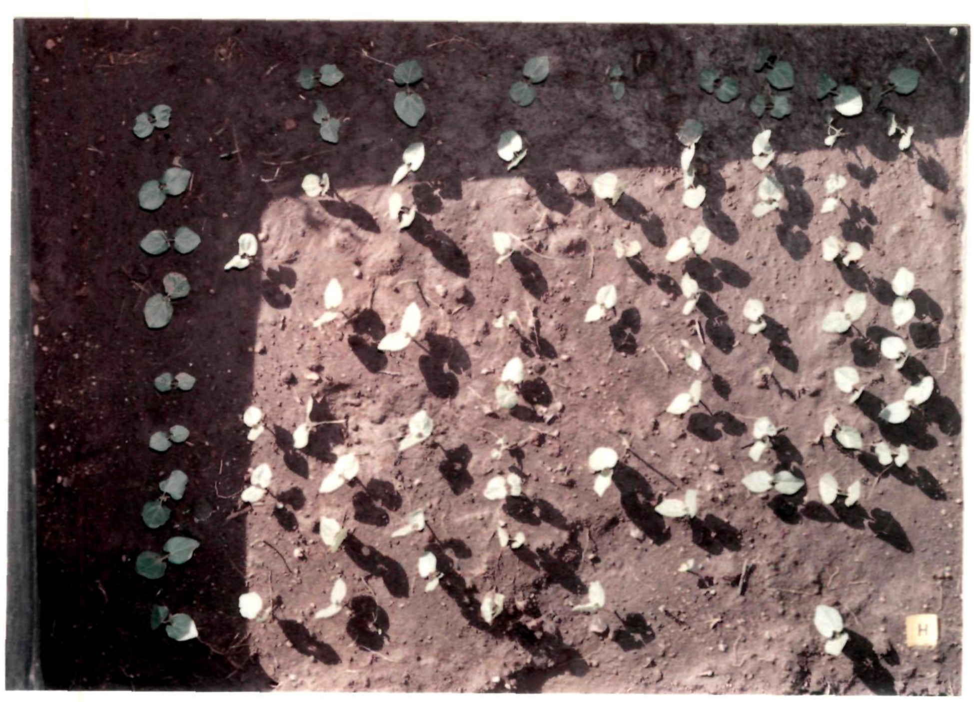

Plate 12: Field emergence test in horsegram (75%)

Plate 13: Standard germination and TZ test in blackgram

1. Normal seedlings (84%)
2. Abnormal seedlings (10%)
3. Dead seeds (6%)
4. TZ treated seeds (germinable: 87%)
   (non-germinable: 13%)

Plate 14: Field emergence test in blackgram (87%)

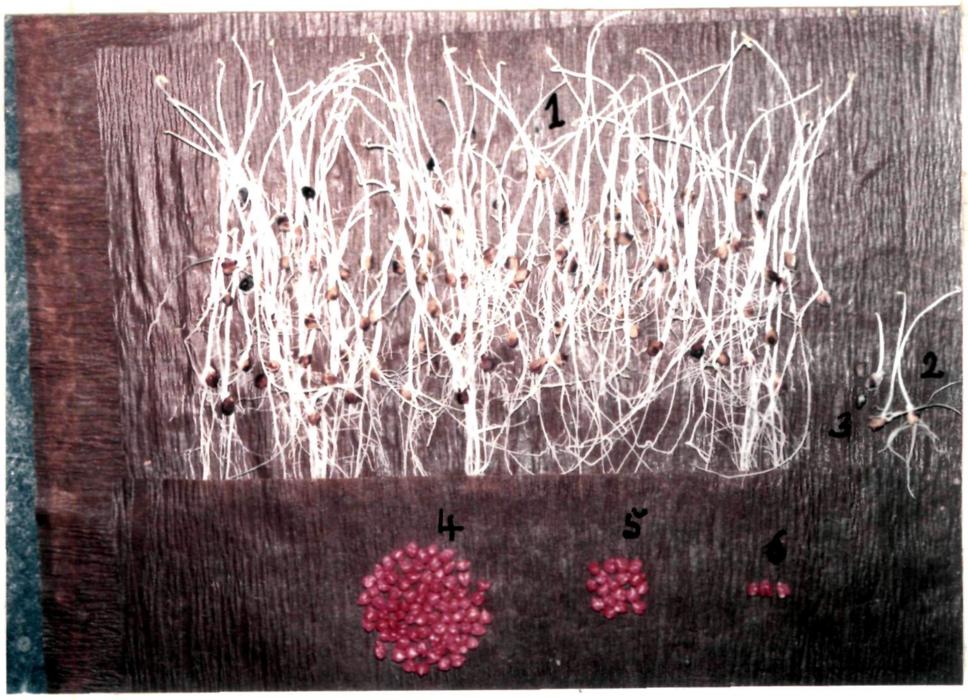

Plate 15: Standard germination and TZ test in bengalgram

1. Normal seedlings (95%)
2. Abnormal seedlings (3%)
3. Dead seeds (2%)
4&5. TZ treated seeds (germinable: 97%)
6. TZ treated seeds (non-germinable: 3%)

Plate 16: Field emergence test in bengalgram (98%)

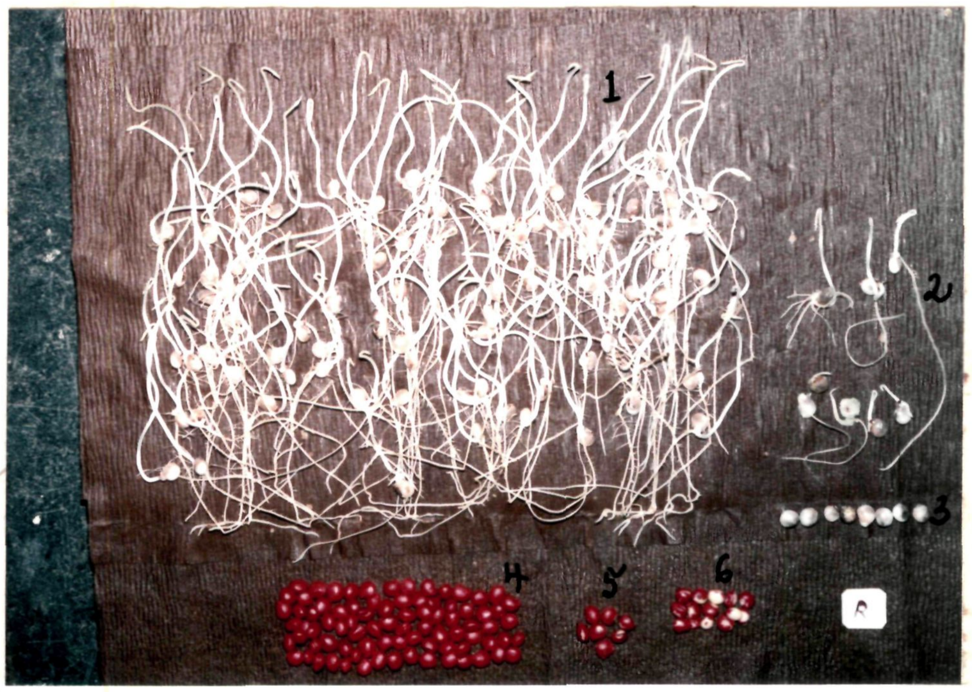

Plate 17: Standard germination and TZ test in redgram

1. Normal seedlings (84%)
2. Abnormal seedlings (8%)
3. Dead seeds (8%)

4 & 5. TZ treated seeds (germinable: 86%)

6. TZ treated seeds (non-germinable: 14%)

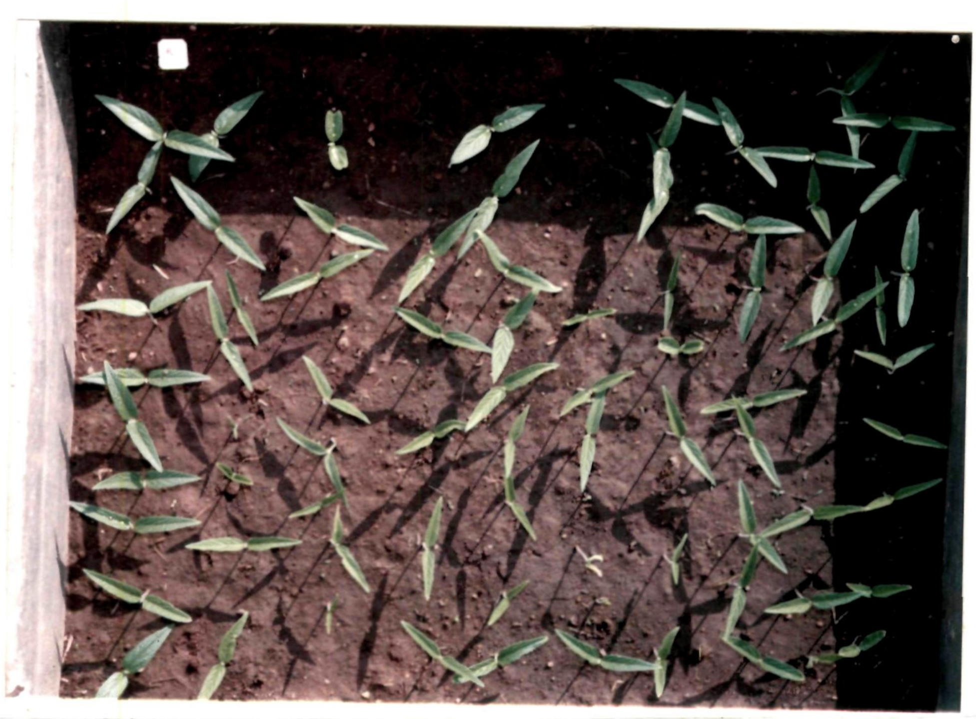

Plate 18: Field emergence test in redgram (76%)

TZ test, standard germination and field emergence tests are presented in Table 1

In 25 °C, the per cent seed viability varied from 87.5 per cent in case of 0.50 per cent dilution to 96.5 per cent in case of 0.75 per cent dilution. In 30 °C, the values ranged from 84.5 per cent in case of 0.75 per cent dilution to 87.5 per cent in case of 0.10 per cent dilution. In 35 °C, the viability values ranged from 86.5 per cent in case of 0.50 per cent as well as 1.00 per cent TZ concentrations to 91.5 per cent in 0.75 per cent concentration. In case of 40 °C, the range was 83.5 per cent in 0.75 per cent TZ dilution to 91.5 per cent in 1.00 per cent TZ dilution.

4.5 Comparative economy of TZ test over standard germination test

4.5.1 Cost analysis

The detailed cost analysis of TZ test and standard germination tests are presented in Table 17, for the six pulse crop seeds. The volume of TZ solution needed to test 200 seeds varied from 14 ml as in case of horsegram to 64 ml in case of redgram (Table 16).

In cowpea, the amount of TZ salt needed to test 200 seeds varied from 0.048 g to 0.480 g depending on the TZ solution concentration and simultaneously the cost increased from Rs. 6.72 to 67.20 from 0.10 per cent dilution to 1.00 per cent dilution (Table 17).

Both in greengram and blackgram, the amount of TZ salt needed to test 200 seeds increased from 0.016 g to 0.160 g from 0.10 per cent dilution to 1.00 per cent dilution respectively and the cost increased from Rs. 2.24 in case of 0.10 per cent dilution to Rs. 22.40 in case of 1.00 per cent dilution (Table 17).

In horsegram the quantity of TZ salt needed to test 200 seeds increased from 0.014 g (0.10% dilution) to 0.140 g (1.00% dilution). Whereas the cost increased from Rs. 1.96 to Rs. 19.60 in case of 0.10 to 1.00 per cent TZ solution concentrations respectively (Table 17).

In bengalgram the quantity of TZ salt needed to prepare 0.10 per cent and 1.00 per cent dilutions for testing 200 seeds varied from 0.060 g to 0.600 g. The cost of the salt varied from Rs. 8.40 in case of 0.10 per cent dilution to Rs. 84.00 in 1.00 per cent dilution (Table 17).

In redgram, to test 200 seeds, the quantity of TZ salt needed varied from 0.064 g to 0.640 g in case of 0.10 per cent and 1.00 per cent concentrations respectively. Similarly the cost increased from Rs. 8.96 in case of 0.10 per cent dilution to Rs. 89.60 in 1.00 per cent dilution (Table 17).

The cost of standard germination test was Rs. 28 for any of the pulse crop seeds being tested which is also presented in Table 17.

Table 16: Total volume of TZ solution needed to test 200* seeds of different pulse crops

Crop	TZ solution (ml)
Cowpea	48.0
Greengram	16.0
Horsegram	14.0
Blackgram	16.0
Bengalgram	60.0
Redgram	64.0

* Minimum number of seeds recommended by Association of Official Seed Analysts (Grabe, 1970)

Table 17: Cost of TZ salt and standard germination test materials used for viability tests in six different pulse crop seeds

Crop	TZ solution concentration (%)	TZ salt needed (g)	Cost (Rs.)
Cowpea	0.10	0.048	6.72
	0.25	0.120	16.80
	0.50	0.240	33.60
	0.75	0.360	50.40
	1.00	0.480	67.20
Greengram	0.10	0.016	2.24
	0.25	0.040	5.60
	0.50	0.080	11.20
	0.75	0.120	16.80
	1.00	0.160	22.40
Horsegram	0.10	0.014	1.96
	0.25	0.035	4.90
	0.50	0.070	9.80
	0.75	0.105	14.70
	1.00	0.140	19.60
Blackgram	0.10	0.016	2.24
	0.25	0.040	5.60
	0.50	0.080	11.20
	0.75	0.120	16.80
	1.00	0.160	22.40
Bengalgram	0.10	0.060	8.40
	0.25	0.150	21.00
	0.50	0.300	42.00
	0.75	0.450	63.00
	1.00	0.600	84.00
Redgram	0.10	0.064	8.96
	0.25	0.160	22.40
	0.50	0.320	44.80
	0.75	0.480	67.20
	1.00	0.640	89.60

Standard germination test (for all the crops)
Total number of seeds used = 400*
Number of replication = 4

Particulars	Quantity (Ns.)	Cost per Unit (Rs.)	Total cost (Rs.)
Germination paper	12	1.50	18.00
Wax sheets	4	2.50	10.00
Grand Total			28.00

* Minimum number of seeds recommended by the International Seed Testing Association (Anon, 1985).

4.5.2 Time analysis

The total test duration of TZ test and standard germination test for 6 pulse crops are presented in Table 18.

The total man hours needed for conducting TZ test and standard germination tests were found to be same (Table 19). In general, the TZ test period decreased with increased TZ solution concentration or increase in incubation temperature as shown in Table 18.

In cowpea, at 25 °C, the test period decreased from 29 to 10 hours 30 minutes in 0.10 per cent and 1.0 per cent TZ solution concentrations respectively. In 30 °C, the test period varied from 24 hours in 0.1 per cent dilution to 10 hours in 1.0 per cent dilution. In 35 °C, the test time decreased from 21 hours in 0.1 per cent dilution to 9 hours 30 minutes in case of 1.0 per cent dilution. In 40 °C, the test period decreased from 19 hours in case of 0.10 per cent TZ solution concentration to 9 hours in case of 1.00 per cent concentration.

In greengram, at 25 °C, the total test duration decreased from 32 hours in 0.10 per cent concentration to 13 hours 30 minutes in 1.00 per cent concentration. At 30 °C, it ranged from 25 hours in case of 0.10 per cent dilution to 10 hours 30 minutes in 1.00 per cent dilution. At 35 °C incubation temperature, the test period ranged from 21 hours in 0.10 per cent dilution to 10 hours 15 minutes in case of

1.00 per cent dilution. At 40 °C incubation temperature, it varied from 13 hours in 0.10 per cent to 10 hours in 1.00 per cent dilution.

In horsegram, at 25 °C, the total test period ranged from 26 to 12 hours 30 minutes in case of 0.10 per cent and 1.00 per cent TZ solution concentrations respectively. At 30 °C, the test period ranged from 24 hours to 11 hour 30 minutes in 0.10 and 1.00 per cent dilutions respectively. At 35 °C, the total test period varied from 21 hours in case of 0.10 per cent to 10 hours 45 minutes in 1.0 per cent dilution. At 40 °C, it varied from 19 hours to 10 hours in 0.10 per cent and 1.00 per cent solution concentrations respectively.

In blackgram, at 25 °C, the test period ranged from 39 to 18 hours in case of 0.10 per cent and 1.00 per cent dilutions respectively. At 30 °C, the test period decreased from 25 hours to 14 hours 15 minutes in 0.10 and 1.00 per cent dilutions respectively. At 35 °C, the test period ranged from 23 hours in 0.10 per cent dilution to 12 hours 30 minutes in 1.00 per cent dilution. Similarly at 40 °C, the test period decreased from 19 hours in 0.10 per cent dilution to 11 hours 15 minutes in 1.00 per cent dilution.

In bengalgram, at 25 °C, the total test period decreased from 45 hours to 20 hours in 0.10 per cent to 1.00 per cent TZ solution concentration respectively. At 30 °C, the test

Table 18: Total duration of TZ test and standard germination tests in six different pulse crop seeds

Crop	TZ solution concentration (%)	Incubation temperature (C) 25		30		35		40	
		hr.	min.	hr.	min.	hr.	min	hr.	min.
	0.10	29	-	24	-	21	-	19	-
	0.25	23	-	17	-	15	-	13	-
Cowpea	0.50	15	-	13	30	13	-	11	-
	0.75	12	-	11	-	10	30	10	-
	1.00	10	30	10	-	9	30	9	-
	0.10	32	-	25	-	21	-	13	-
	0.25	22	-	15	30	15	-	11	45
Greengram	0.50	16	30	13	30	12	-	10	45
	0.75	15	-	12	-	11	-	10	15
	1.00	13	30	10	30	10	15	10	-
	0.10	26	-	24	-	21	-	19	-
	0.25	20	-	18	-	16	-	12	30
Horsegram	0.50	18	-	16	-	14	-	11	-
	0.75	14	-	13	-	11	15	10	15
	1.00	12	30	11	30	10	45	10	-
	0.10	39	-	25	-	23	-	19	-
	0.25	32	-	23	30	20	-	14	45
Blackgram	0.50	29	-	18	-	15	15	13	-
	0.75	22	-	16	30	13	45	11	45
	1.00	18	-	14	15	12	30	11	45
	0.10	45	-	30	-	22	-	15	15
	0.25	38	-	24	-	19	-	13	30
Bengalgram	0.50	34	-	21	-	17	15	12	15
	0.75	22	-	18	-	15	-	11	45
	1.00	20	-	15	45	13	45	11	-
	0.10	25	-	22	-	18	-	17	-
	0.25	21	-	19	-	16	30	15	-
Redgram	0.50	19	-	17	-	15	-	14	-
	0.75	16	-	15	-	13	30	12	30
	1.00	14	-	13	-	12	-	11	30

Standard germination test period (Days for final count)
(Anon., 1985)

Cowpea	:	8
Greengram	:	7
Horsegram	:	7
Blackgram	:	7
Bengalgram	:	8
Redgram	:	10

Table 19:Comparative manhours needed for TZ test and standard germination tests in six different pulse crops

Test	Steps	Time needed (minutes)
TZ test*	i) Preparation	30 to 45
	ii) Evaluation	30 to 45
Standard germination test**	i) Putting seeds for germination	30 to 45
	ii) Taking counts	30 to 45

* 200 seeds (Grabe, 1970)
** 400 seeds (Anon, 1985)

period ranged from 30 hours to 15 hours 45 minutes in 0.10 and *1.00* per cent *dilutions respectively. At 35 °C, the test* period varied from 22 hours in 0.10 per cent dilution to 13 hours 15 minutes in 1.00 per cent dilution. At 40 °C, it reduced from 15 hours 15 minutes to 11 hours in 0.10 and 1.00 per cent dilutions respectively.

In case of redgram, at 25 °C, the test period decreased from 25 hours in 0.10 per cent dilution to 14 hours in 1.0 per cent dilution. At 30 °C, it decreased from 22 hours in 0.10 per cent to 13 hours in case of 1.00 per cent dilution. At 35 °C, the test period ranged from 18 hours in 0.10 per cent dilution to 12 hours in 1.00 per cent dilution. At 40 °C, the test period decreased from 17 hours in case of 0.10 per cent solution concentration to 11 hours 30 minutes in case of 1.00 per cent solution concentration.

The standard germination test required minimum of 7 days in case of greengram, horsegram and blackgram and up to days in case of redgram.

DISCUSSION

V. DISCUSSION

Study of seed viability is important in determining the planting value of seeds. Testing seeds for germination by conventional methods takes longer time, but many a time viability of seeds is required to be known within a day or two. Under such situations, one must resort to some other method for assessing seed viability like that of tetrazolium test (Agrawal, 1994). Tetrazolium testing is one of the quick viability tests which can be used for testing both the dormant and non-dormant seed lots. It is also used for knowing the cause of seed deterioration (Patil and Dadlani, 1993). In the present investigation, the efficacy of different concentrations of tetrazolium salt solution in evaluating the seed viability of six major pulse crop seeds has been compared with standard germination and field emergence test results which are discussed under the following headings.

1. Time required for preconditioning the seeds for TZ test.
2. Time required for TZ reaction.
3. Standard germination and field emergence test results.
4. Comparison of TZ test results with that of standard germination test.
5. Comparison of TZ test results with that of field emergence test.
6. Economics of TZ test over standard germination test.
7. Practical application of the present investigation and
8. Future line of work.

5.1 Time required for preconditioning the seeds for TZ test

Time needed for preconditioning or soaking period depends on the seed characteristics (Grabe, 1970), age of the seed lot and temperature at which the seeds are soaked (Patil and Dadlani, 1993).

Among the six pulses, cowpea seeds have taken less time (6 hours) by virtue of its soft seed coat. Greengram, horsegram and bengalgram have taken slightly more time (7 hours) whereas blackgram and bengalgram have taken longer time (8 hours) for preconditioning due to increased number of hard seeds and thick seed coat respectively. The above statements of Grabe (1970) are in agreement with these findings. All the above tests have been conducted at room temperature ($25 \pm 1^{o}C$).

Pasha and Das (1982) soaked four replicates of 100 seeds of soybean in distilled water for four hours before pealing-off the seed coat during the conduct of TZ test for seed viability. Ellis et al. (1985) suggested 18 hours of imbibition or soaking for *Cicer arietinum* L., seeds and in case of *Vigna* sp., 22 hours soaking has to be followed before pealing off the seed coat for TZ treatment. During preconditioning, the priority was given for saving time so as to reduce the total time required for TZ testing. Hence, the minimum time at which easy pealing of seed coat could be obtained has been taken into consideration for further studies (Table 1). For preparing the seeds for TZ staining reaction, the seed coats were pealed off after sufficient soaking at room temperature ($25 \pm 1^{o}C$) so as to expose the embryo and other essential structures for TZ staining as prescribed by Grabe (1970) and Ellis et al. (1985).

5.2 Time required for TZ reaction

Staining of seed tissues by Tetrazolium is influenced by temperature, TZ solution concentration, duration of incubation in TZ solution and the seed characteristics (Grabe, 1970).

As a rule of thumb, in all the six pulse crops studied, within a given temperature of incubation, as the concentration of tetrazolium solution increased, the staining period decreased and similarly within a given TZ solution concentration, as the temperature of incubation increased, the staining period gradually decreased. The staining period drastically reduced in case of higher temperature of incubation and higher concentration of TZ solution combinations viz., $40^{o}C$ and 1.0 per cent TZ solution as compared to the lowest temperature and TZ solution concentrations ($25^{o}C$ and 0.10% concentration).

In cowpea, the TZ reaction time needed was 22 hour at $25^{o}C$ and 0.10 per cent TZ solution dilution; whereas it reduced to two hours at $40^{o}C$ and one per cent TZ solution. In greengram, TZ reaction time was 24 hours at 0.10 per cent dilution and $25^{o}C$ incubation temperature. The TZ reaction time reduced to two hours 40°C temperature and 1.00 per cent TZ solution. In case of Horsegram, the TZ reaction time reduced from 18 hours at 25°C and 0.10 per cent TZ solution concentration to two hours at 40°C and 1.0 per cent TZ solution concentration. In Blackgram, the TZ reaction time was 30 hours at 25°C temperature and 0.10 per cent TZ solution concentration whereas the time reduced to two hours 15 minutes at 40°C and 1.00 per cent TZ solution. In bengalgram the TZ reaction time was reduced from 36 hours in case of 25°C temperature and 0.10 per cent dilution to two hours in case of 1.00 per cent TZ solution with incubation temperature of 40°C. In redgram, the TZ reaction time was reduced from 17 hours in 0.10 per cent TZ solution concentration with 25°C incubation temperature to three hours 30 minutes at 40°C temperature and 1.00 per cent TZ solution concentration. The above results are supported by the views of Grabe (1970) and Ellis et al. (1985), who opined that the dehydrogenase activity triggered with increase in incubation temperature.

5.3 Standard germination and field emergence test results

In case of cowpea, the standard germination test values were close to field emergence by 3.5 per cent. In greengram and blackgram, the standard germination test result was 4.75 per cent more than the field emergence value. In horsegram, both the test values differed by 0.25 per cent (Table 3). In case of bengalgram, the field emergence values were slightly higher than standard germination values (by 2.75%). The reason could be due to field emergence test of bengalgram was conducted in winter season and since it is a winter pulse crop it gave slightly higher value compared to standard germination test performed at 25 °C. This difference could have been avoided if the germination test was conducted at 20 °C constant temperature or 20 °C to 30 °C alternating temperature as prescribed in I.S.T.A. rules (Anon, 1985). In redgram, there was a difference of 14.25 per cent between standard germination and field emergence test values (Table 3), which could be attributed to the difference resulted due to lower seed vigour.

The above findings could be suported by the views of Erickson and Porter (1938) who conducted field emergence test in Alfalfa and Redclover, wherein the seedling emergence was considerably less than laboratory germination as in case of redgram in the present study and that the higher germinating lots responded well in the field like in case of bengalgram during the present study.

If standard germination test is conducted under standardized controlled conditions for seed germination when field conditions at planting are near optimum, the standard germination test results correlated very well with field emergence (Perry, 1977; Egli and Tekrony, 1979). These findings could be compared with results of cowpea, greengram blackgram and horsegram in the present study.

5.4 Comparison of TZ test results with that of standard germination test

Gupta and Raturi (1975) described that 1 to 4 categories were found to be the most feasible viable categories in Cassia fistula and Cassia nodosa seeds at the time of conducting TZ test. In a similar way, during the conduct of present investigation the categories 1 to 7 were found to be the most feasible ones in cow pea out of 10 categories, 1 to 13 out of 17 in greengram; 1 to 11 out of 16 in horsegram, 1 to 9 out of 13 in black gram; 1 to 10 out of 13 in bengalgram and redgram were found to be the most feasible category combinations which gave the results very close to that of germination test values.

The validity of the above test can be further confirmed by 't' test, wherein these category combinations have given the minimum t values compared to other category combinations. This was described by the work of Pasha and Das (1982) who conducted TZ test in soybean seeds. They added the categories until the value nearest to the germination percentage, wherein these categories were expected to form viable categories. To arrive at the correct conclusion, a 't' test was applied to various category combinations and these combinations exhibiting the lowest 't' value were taken to be the most feasible ones. The table 't' value (P = 0.05) was taken as reference for comparing the minimum 't' values obtained in different TZ treatments. The total percentage values of the categories in these combinations were compared with the actual germination percentage.

In cowpea the different TZ treatment combinations estimated standard germination within the range of 1.0 to 6.5 per cent (Table 5). The 't' test indicated that in TZ treatments of 25 °C with 0.10 per cent TZ solution; 40 °C incubation temperature with 0.75 and 1.00 per cent TZ solution dilutions, there were significant differences between TZ test and standard germination test results, indicating that very low and very high TZ solution concentrations are not feasible to use in case of cowpea. The findings of Grabe (1970) support the above results, who opined that either high or low temperatures as well as concentrations may give variable results due to feable staining (low temperature / concentration) or overstaining (high temperature / concentration).

In greengram there was a difference of 2.75 to 5.75 per cent between different TZ treatments and standard germination test results (Table 7). The treatment differences were found to be non-significant, indicating that all the TZ treatment results are very close to standard germination test results.

In case of horsegram the difference was $\pm$0.75 to 9.25 per cent (Table 9); in blackgram it was $\pm$ 4.75 to 5.75 per cent (Table 11) and in bengalgram it was 2.75 to 3.75 per cent (Table 13) and finally in redgram the difference was within 5.25 to 7.75 per cent (Table 15). The above results are similar to the results of parallel studies like in case of pea seeds, Laszlo (1972) reported that a close correlation was found between TZ test and germination capacity. Rytko et al. (1985) stated that the germination capacity and viability of wheat, rye and barley seeds were one to two per cent higher when estimated by TZ test than by standard germination test, and that the difference was most marked in the seeds of low sowing value. But Mason et al. (1982) reported that in case of mechanically damaged soybean seeds, TZ test indicated a significantly higher percentage germination than did the standard germination.

In almost all the crops that were tested during the present investigation, the TZ treatments gave viable seed per cent very close to standard germination which was confirmed by applying 't' test. The TZ treatment differences were found to be non-significant in all the crops (except in extreme temperature/concentration treatments of cowpea) indicating that the TZ solution concentrations of 0.1 to 1.0 per cent can be satisfactorily used for testing the seeds in the incubation temperatures of 25, 30, 35 and 40 ^{o}C. Thus it can be used as an alternative test for assessing the seed viability of pulse crops instead of standard germination test whereby one can economise the viability test in terms of time taken for testing as well as the cost of testing.

5.5 Comparison of TZ test results with that of field emergence test

In case of cowpea, the TZ test estimated field emergence with ± 3 to 4.5 per cent wherein the standard germination test has predicted it within 3.5 per cent (Table 5). In greengram TZ test and standard germination test estimated field emergence within ±0.5 to 7.5 per cent and 4.75 per cent respectively (Table 7). Similarly in horsegram TZ test and germination test estimated field emergence within the range of 0.5 to 13.5 per cent and ±0.25 per cent respectively (Table 9). In blackgram TZ test predictedfield emergence within ±1.0 to 9.5 per cent whereas standard germination predicted it by 4.75 per cent (Table 11).

In bengalgram the field emergence has been predicted to the accuracy of within ±0.5 to 5.5 per cent by the TZ test, whereas standard germination test predicted it within ±2.75 per cent (Table 13).

In case of redgram, both the TZ test and standard germination tests overestimated field emergence by 6.5 to 19.5 per cent and 14.25 per cent respectively (Table 15). Such a deviation in test results either between germination test and field emergence or between TZ test and field emergence had been reported by several workers and Mason et al. (1982) found that in mechanically damaged soybean seeds the standard germination test estimated average field emergence within ±12.0 per cent whereas TZ test estimated it by zero to 18 per cent. Rytko et al. (1985) stated that such larger differences was most marked in the seeds of low sowing value. Erickson and Porter (1938) reported that the emergence of seedlings in field was considerably less than laboratory germination in case of Alfalfa and Redclover and the higher germinating lots responded best in the field.

5. 6 Economics of TZ test over standard germination test

Moore (1962,1973) has described the importance of TZ test for evaluating seed soundness in many of the seed types. Grabe (1970), Patil and Dadlani (1993) mentioned that TZ test is one of the most promising quick tests of viability. The economic analysis of the TZ test in the six major pulse crop seeds during the present study have been discussed hereby.

1. Cowpea: for standard germination test, the material cost was 28 Rupees (for 400 seeds). The present study reveals that we can use TZ solutions of either 0.1 per cent or 0.25 per cent to test the viability, whose cost is still lesser than standard germination test materials (Table 17).

As far as testing time is concerned, one can use higher concentration of TZ salt solutions in combination with the higher temperature of incubation whereby one can test the viability within a day whose time economy cannot be compared with that of standard germination test since it takes eight days (Table 18).

<u>Greengram</u>: In case of greengram, one can use any TZ solution concentration for testing, since all the concentrations are economical to use compared to that of standard germination test (Table 17). Thus, in terms of both time (Table 18) and cost TZ test is economical than germination test in greengram.

<u>Horsegram</u>: In horsegram it is clear from table 17 that even if one uses 1.0 per cent TZ solution it will be economical to use TZ than standard germination test. The TZ test at 1.0 per cent concentration with 40 °C incubation temperature taken 10 hours, but standard germination test required seven days time for testing (Table 18).

<u>Blackgram</u>: In blackgram also it is economical to use all the TZ concentration ranges from 0.1 to 1.0 per cent when compared to the standard germination test (Table 17). Again in terms of time factor, [illegible], TZ test was far beyond comparison since the standard germination test required seven days (Table 18).

<u>Bengalgram</u>: Here it is economical to use 0.10 per cent and 0.25 per cent TZ solution compared to standard germination test cost (Table 17) and if one needs to test the viability within a day, it is better to go for higher TZ solution concentration by neglecting the cost (Table 18).

Redgram: In redgram again the use of 0.10 and 0.25 TZ solutions found to be economical as far as cost factor is concerned when compared with the standard germination test (Table 17). and if the viability assessment is required in few hours, one can advocate higher TZ concentrations at higher temperatures (Table 18).

5.7 Practical application of the present investigation

TZ test can be used in pulse crop seeds for seed testing and seed research programmes. During the present investigation the topographical staining patterns have been standardized for cowpea, greengram, horsegram, blackgram, bengalgram and redgram for evaluating viable seeds after TZ staining; which can be used as reference by those who need to apply this test for assessing viability of any other pulse crop seed.

It is an important fact that TZ test is highly economical to use in all the concentration ranges (0.1 to 1.0 %) in case of small seeded pulse crops like horsegram, blackgram and greengram compared to standard germination test cost.

It is easy to estimate the viability of using TZ salt if in case there is more hard seed content occurs in a given seed lot; eg. blackgram, horsegram, etc., when compared to standard germination test.

In almost all the crops tested, TZ results were almost on par with the standard germination test results and thus it can be used as one of the alternative tests to standard germination test to get the results in a very short span of time.

In pulses they take minimum of one week for standard germination test which is really time consuming, whereas by using TZ test one can get the viability test result within 10 or 11 hours at the highest concentration (1.0%) and higher treatment temperature (40 ^{o}C).

Thus the results of the present study can be satisfactorily used by seed testing labs, seed researchers, plant breeders, seed companies and seed traders for quick viability studies.

5.8 Future line of work

1. Efficacy of TZ test in the temperature of below 25 ^{o}C and above 40 ^{o}C.

2. Study of TZ staining patterns in differentially aged seed lots and its comparison with standard germination and field emergence test results.

3. Effect of different TZ salt solutions on the storability of TZ solution.

4. Effect of different soaking temperature on the preconditioning time and the adverse effects of higher temperature on the accuracy of the test.

5. Efficacy of different brand of TZ chemicals on seed testing

6. Effect of slow imbibition vs direct soaking in water on the accuracy of TZ test results.

7. Study of variation in TZ test results in case of treated seeds.

SUMMARY

VI. SUMMARY

An investigation on the efficacy of tetrazolium test for evaluating seed viability of six major pulse crops was conducted in comparison with standard germination and field emergence tests at the Department of Seed Technology, Agricultural College, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore, with the objectives of standardizing the preconditioning time, studying the effects of different TZ solution concentrations and incubation temperatures on the accuracy of TZ test results. During the study the efficacy of TZ test was compared with standard germination and field emergence test and emphasis was also made to compare TZ test economy with that of standard germination.

Seeds of six major pulse crops, viz., cowpea (C-152), greengram (local), horsegram (local), blackgram (T-9), bengalgram (local) and redgram (Hyd.3C) were collected from different sources at Bangalore, all of them were about six to eight months old lots. They were tested for initial moisture content, germination and seed health. They were stored in polythene covers for further studies. All the six pulse crop seeds were tested for the soaking time required for preparing seeds for TZ treatment, since it varies from crop to crop.

The TZ solution was prepared using tap water having neutral pH. The TZ solution of different dilutions viz., 0.10, 0.25, 0.50 and 0.75 per cent solution were prepared from 1.00 per cent TZ solution.

After standardizing the preconditioning time for each crop, the TZ treatment time was standardized crop wise for different incubation temperatures viz., 25, 30, 35 and 40 ^{o}C for different TZ dilution treatments. The TZ treatment duration was also standardized before the main research work.

In the main study, in each crop, in total 200 seeds were taken for each treatment . After preconditioning and removal of seed coat, the seeds were treated with one of the five TZ solution dilutions and incubated at one of the four temperatures studied. The stained seeds were latter rinsed in water two to three times to remove the traces of TZ chemical and then stained seeds were kept in water in a petri dish to avoid drying. Based on topographical staining patterns, the seeds were classified into different categories. The above procedure was repeated for all the treatment combinations of TZ dilution and incubation temperature for all the six pulses crop seeds. For all the tests, the completely Randomized Design was applied. Based on the least 't' values for the category combinations, seed categories were classified as 'Germinable' and 'Non-germinable'. The per cent seed viability as assessed by TZ test was compared with the results of standard germination and field emergence tests.

Since the TZ test is one of the most reliable quick test of seed viability its efficiency was compared with standard germination test in terms of test results, test economy and test duration. The results of the investigation are summarized as below:

1. The period of preconditioning varied from six hours in case of cowpea to eight hours as in case of bengalgram and blackgram and thus the period of preconditioning varied from crop to crop, depending on seed coat/seed characteristics and temperature of water during soaking.

2. The period of TZ staining treatment is also a variable factor, which depends on the crop species, vigour level of seeds and especially the concentration of the TZ solution and incubation temperature. The TZ treatment period increased from lowest temperature / TZ solution concentration ($25^{o}C$/0.1 %) to the highest temperature/TZ solution concentration ($40^{o}C$/1.0 %). In cowpea and greengram it ranged from 2 to 22 hours; in horsegram it ranged from 2 to 18 hours; in blackgram from 2 hours 15 minutes to 30 hours; in bengalgram from 2 hours to 36 hours and in redgram it varied from three hours 30 minutes to 17 hours.

3. The percentage viable seeds as determined by TZ test was compared with standard germination test at five different TZ dilutions and four different incubation temperatures. The results indicated a close relationship between TZ test and standard germination test as far as seed viability assessment is concerned. In cowpea, among the various treatment combinations, TZ test estimated standard

germination test results within ±6.5 per cent, in greengram it was within ±5.75 per cent, in horsegram, within ±9.25 per cent, in blackgram within ±5.75 per cent and in case of bengalgram, it was within ± 3.25 per cent. In redgram the TZ test predicted standard germination test result within ±7.75 per cent. The above results revealed that TZ test can predict standard germination test results within very close range.

4. The study has also been extended to compare the TZ test results and standard germination test results with field emergence tests in all the six pulse crops. The standard germination test estimated field emergence of cowpea within ±3.0 per cent. Whereas TZ test estimated it within ±6.5 per cent. In greengram, the standard germination and TZ tests predicted field emergence within ±4.75 and ±7.5 per cent respectively. In horsegram standard germination and TZ tests estimated field emergence within ±0.25 and ±9.00 per cent respectively. In blackgram standard germination and TZ tests predicted field emergence accurately within ±4.75 and ±9.5 per cent respectively. In bengalgram, the standard germination and TZ test estimated field emergence within ±2.75 and ±5.5 per cent respectively. In case of redgram, the standard germination and TZ tests predicted field emergence within ±14.25 and ±19.5 per cent respectively. The greater differences between lab test and field emergence values are attributed to vigour level of seed lots. The above results

indicate that TZ test estimated field emergence in a similar way the standard germination test did it.

5. TZ test economy was compared with that of standard germination test in terms of cost as well as the time required for conducting the test. In small seeded pulse crops like blackgram, greengram and horsegram, TZ test was found to be economical than standard germination test among all the concentration ranges up to 1.00 per cent dilution. Whereas, in bigger sized pulse crop seed the TZ cost did not exceed even up to the concentration levels of 0.25 per cent dilution as in case of cowpea, bengalgram and redgram. As far as total manhours for the test is concerned, both TZ test and standard germination test required approximately one hour 30 minutes each, but the fact that the analyst who conducts TZ test should have thorough knowledge of TZ reaction and evaluation compared to standard germination test. As far as the total test duration is concerned, TZ test can be performed within nine hours in cowpea (40^{o}C/1.00%), whereas the standard germination test needed eight days. In greengram, TZ test could be terminated in 10 hours (40^{o}C/1.00%), but standard germination test needs minimum of seven days. In horsegram, the minimum time needed for TZ test was 10 hours (40^{o}C/1.00%), whereas for standard germination the test period needed was 7 days. In case of blackgram the TZ test can be performed within 11 hours 15 minutes (40^{o}C/1.00%), whereas standard germination test

needed seven days for completion of test. In bengalgram, TZ test can be conducted in 11 hours (40 °C/1.00%), but the standard germination test required eight days for the viability assessment. In case of redgram, TZ test could be able to predict seed viability in 11 hours 30 minutes (40 °C/1.00%), but the standard germination test required 10 days to get the final result. Thus, TZ test can be used in place of standard germination test in pulse crops to get quick estimates of seed viability.

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VII. REFERENCES

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* Original not seen

APPENDICES

APPENDIX I : Initial germination, moisture content and seed health test in six different pulse crop seeds

Crop	Germination (%)	Moisture content (%)	Per cent infection
Cowpea	94.00	10.23	0.0
Greengram	95.00	12.49	0.0
Horsegram	79.00	10.68	2.0 (*Rhizopus* sp.)
Blackgram	91.00	9.49	1.5 (*Rhizopus* sp.)
Bengalgram	96.50	11.23	2.2 (*Aspergillus* sp.)
Redgram	92.50	10.21	0.0

Appendix II: Number of days recommended for first and final counts in standard germination test for the pulse crop seeds (Chalam *et al*., 1967; Anon., 1985)

Crop	I Count (days)	Final count (days)	Additional directions to to break the dormancy
Cowpea	5	8	-
Greengram	5	7	-
Horsegram	4	7	-
Blackgram	4	7	-
Bengalgram	5	8	-
Redgram	4	10	-

APPENDIX III: Preparation methods for obtaining different TZ solution concentrations

TZ solution concentration (%)	Proportion of 1.0 % TZ solution used (parts)	Proportion of water added (parts)
0.10	1	9
0.25	1	3
0.50	1	1
0.75	3	1